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3792

Blood Chemistry in Intestinal Obstruction.

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The work of Tileston and Comfort,[1] Whipple and his associates,[2] Mac Callum,[3] Hastings,[4] Haden and Orr,[5] and others has demonstrated that obstruction in the upper intestinal tract produces characteristic changes in the blood chemistry. These may be summarized as follows: a decrease in the concentration of chlorides, a late increase in non-protein and urea nitrogen, and an increase in the carbon dioxide combining power of the blood plasma. As a result of a number of experiments reported by L. R. Dragstedt,[6] C. A. Dragstedt,[7] P. R. Cannon,[8] and their associates from 1916 to 1921, these authors concluded that the toxemia of intestinal obstruction was due to the absorption of chemical fractions produced by the proteolytic activity of the intestinal bacteria and that the factor permitting their absorption was circulatory damage to the intestinal mucosa as a result of distention. In the present experiments determinations were made of the non-protein nitrogen, urea nitrogen, and chloride concentration in the blood of dogs subjected to the various procedures described in the early reports. The following points are brought out.

(1). The production of isolated closed segments of the lower duodenum or upper jejunum, causes a toxemia similar to that resulting from obstruction at the same level and roughly proportionate to the degree of toxemia, a fall in the blood chlorides, and a terminal rise in non-protein and urea nitrogen.

(2). The toxemia and decrease in blood chlorides is in turn dependent upon distention of the closed intestinal segment. If this distention is relieved by aspiration of the fluid within the segment by a needle thrust through the abdominal wall, the toxemia is relieved and there occurs a proportionate return of the blood chloride concentration toward the normal level.

(3). The intra-peritoneal injection into normal dogs of the fluid aspirated from such closed segments produces a toxemia accompanied by a fall in blood chlorides, and a rise in non-protein and urea nitrogen.

(4). If isolated segments of the lower duodenum and upper jejunum are left open and permitted to drain freely into the abdominal cavity, many dogs will not develop peritonitis and will continue to live for long periods in good health. Such animals show no significant changes in the blood chemistry. It seems evident that the substances responsible for the toxemia and accompanying fall in blood chlorides and rise in non-protein and urea nitrogen are not present in the fresh secretions of the duodenal or jejunal mucosa.

(5). After varying periods of from 2 weeks to 2 months, a second operation has revealed that these draining segments may become sterilized. In such animals, with sterile segments of the upper jejunum, a ligation of the mesenteric blood supply to the segment and resultant complete autolysis, has not produced any adverse symptoms nor the usual changes in the blood chemistry.

(6). On the other hand, ligature of the blood supply to a similar segment that has not been sterilized by drainage, causes a profound toxemia, fall in blood chlorides and rise in non-protein and urea nitrogen, and death in 24 to 48 hours.

(7). It is significant that histamine, which may be produced by the action of intestinal bacteria from histidine, has been found in the fluid of closed intestinal loops and in the obstructed intestine, by both the method of biological assay and chemical analysis, in amounts sufficient to account for an appreciable part of the toxicity of these fluids. The subcutaneous injection of histamine in dogs produces symptoms of depression not unlike those following injection of obstruction fluids, a similar splanchnic engorgement, a fall in blood chlorides, and a rise in non-protein and urea N.

(8). The toxic fractions in obstruction fluids are powerful secretagogues, and when injected into dogs cause a marked augmentation of gastric, pancreatic, and intestinal secretion. Accordingly it seems probable from this as well as other evidence obtained by other workers, that the fall in blood chlorides in obstruction is due to their accelerated passage into the alimentary tract and failure of reabsorption.

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[1] Tileston, W., and Comfort, C. W., *Arch. Int. Med.*, 1914, xiv, 620.

[2] Whipple, G. H., Rodenbaugh, F. H., Kilgore, A. R., *J. Exp. Med.*, 1916, xxiii, 123; Cooke, J. V., Rodenbaugh, F. H., and Whipple, G. H., *J. Exp. Med.*, 1916, xxiii, 717.

[3] MacCallum, W. G., Lintz, J., Vermilye, H. N., Leggett, T. H., and Boas, E., *Bull. Johns Hopkins Hosp.*, 1920, xxxi, 1.

[4] Hastings, A. B., Murray, C. D., and Murray, H. A., *J. Biol. Chem.*, 1921, xlvi, 223.

[5] Haden, R. L., and Orr, T. G., *J. Exp. Med.*, 1923, xxxvii, 365; Dragstedt, L. R., Moorhead, J. J., and Burcky, F. W., PROC. SOC. EXP. BIOL. AND MED., 1916 xiv, 17, and *J. Exp. Med.*, 1917, xxv, 421.

[6] Dragstedt, L. R., Dragstedt, C. A., McClintock, J. T., and Chase, C. S., *J. Exp. Med.*, 1919, xxx, 109.

[7] Dragstedt, C. A., and Moorhead, J. J., *J. Exp. Med.*, 1917, xxvii, 359; Dragstedt, C. A., Dragstedt, L. R., Chase, C. S., *Am. J. Physiol.*, 1918, xlvi, 366.

[8] Cannon, P. R., Dragstedt, C. A., and Dragstedt, L. R., *J. Infect. Dis.*, 1920, xxvii, 139.

3793

Comparative Sensitiveness of Blood Pressure and Intestinal Motility to Epinephrine.

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General anesthetics have long been known to depress motility of the gastrointestinal tract. This has recently been studied in some detail by Miller,[1] who reports that ether and chloroform have a very marked depressant effect. We have previously reported that the hemodynamic effect of epinephrine is materially altered by general anesthetics (ether).[2] Since previous studies on the comparative effect of epinephrine upon blood pressure response and gastrointestinal motility[3, 4] have been made on animals under general anesthesia it seemed advisable to repeat this on the unanesthetized animal.

Dogs were used. Intestinal motility was recorded from fistulae of the jejunum by the balloon method. Carotid blood pressure was recorded from arteries previously transplanted externally to the skin. Epinephrine was injected intravenously by means of a Woodyatt pump for varying intervals of time from 2 to 10 minutes. Simultaneous records of blood pressure and intestinal motility have been obtained from 4 animals to whom neither anesthetics nor any drugs have been administered for 24 hours. In each instance a rise of blood pressure has been seen in the absence of intestinal inhibition. Simultaneous records have also been obtained in 6 animals to whom morphine has been administered, and in 1 animal to whom paraldehyde has been given. In each of the animals a rise of blood pressure was obtained without intestinal inhibition. In every instance a sufficient dose of epinephrine was effective in maintaining intestinal inhibition.

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[1] Miller, G. H., *J. Pharm. and Exper. Ther.*, 1926, xxvii, 41.

[2] Dragstedt, C. A., and Wightman, A. H., PROC. SOC. EXP. BIOL. AND MED., xxv, 22.

[3] Hoskins, R. G., and McClure, C. W., *Am. J. Physiol.*, 1912, xxxi, 59.

[4] Durant, R. R., *Am. J. Physiol.*, 1925, lxxii, 314.

3794

Bartonella Infection in Local Rats.

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Mayer, Borchardt and Kikuth[1] have shown that the anemia which develops in white rats after splenectomy is due to the activation of a latent infection with microorganisms belonging to the group *Bartonella.* For these microorganisms, they use the name *Bartonella muris.* The infection is general among the rats in Hamburg. There is a severe, acute form to which the rats succumb within a few days, and, a milder form from which they may recover spontaneously or which leads to death within several weeks. The spontaneous disappearance of the parasites may be followed later by a fatal or non fatal recidive.

Lauda[2] was able to confirm the observations of Mayer, Borchardt and Kikuth on Viennese rats although the infection was not as general as it was in Hamburg. Nauck[3] obtained positive results in Peking. Rats from southern Italy showed no *Bartonella* in their blood after removal of the spleen. When injected with the organs of Viennese rats before the splenectomy, they developed a typical *Bartonella* anemia after the operation.[4]

Since rats are so widely used for experimental studies, an examination of local rats for *Bartonella muris* is worth while. Two strains of rats were at our disposal. One was from a standard

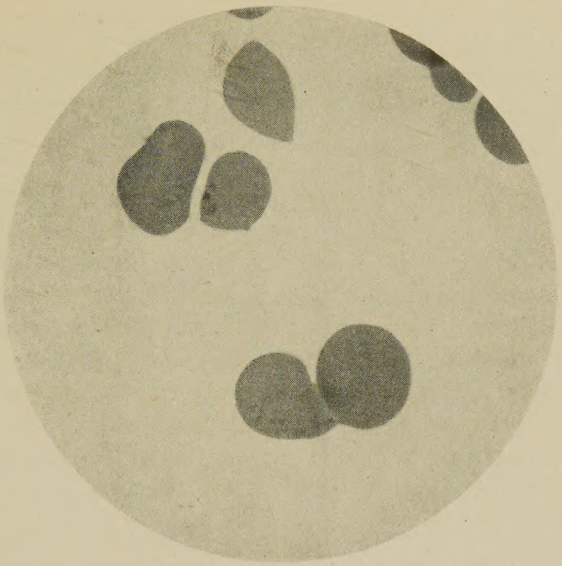

FIG. 1. *Bartonella Muris.*
Note the parasites overlapping the edges of the erythrocytes.
Modification 1500 times.

pedigreed stock kept in the laboratories for nutritional studies and the other one was obtained from a local dealer and much cheaper than the first strain.

Of the pedigreed rats, all which have so far been tested have proved infected. The *Bartonella* appeared in the peripheral blood from 4 to 5 days after the splenectomy. Within 2 days the red count then dropped from 6½ millions to 2½ millions and the hemoglobin content decreased from 100% to 35%. There was a marked neutrophile leukocytosis. Some of the rats died during the severe attack of anemia; some recovered spontaneously. Recovery starts with a sudden and very marked shower of normoblasts.

A very interesting observation has been made on young rats of this group, aged about 4 weeks. Their blood already contained numerous *Bartonellae* before the operation. In normal adult rats it is very difficult to detect the parasites. If present, they are very scanty (Lauda). The results with the rats of the second group were different. Only 1 rat out of 7 became anemic. It recovered completely within 3 weeks. Two of the splenectomized rats were starved for 3 days. They, too, showed no *Bartonella*. One rat died from an hemorrhagic-purulent-broncho-pneumonia. There were no *Bartonellae* in the heart's blood.

For the detection of the microorganisms the panoptic blood staining method of Pappenheim gives very good results. We use a slightly alkaline Giemsa's solution. Short decolorizing of the stained films in 1% $NaCO_3$ solution brings out the *Bartonella* most distinctly. It is helpful especially if there are only very few parasites present.

The *Bartonella* is first visible free between the red blood cells. It is a small biscuit shaped body composed of two granules kept together by a thin capsule. Later, the bodies become attached to the surface of the erythrocytes. They now have the shape of a slender rode that stains blue and contains two small purple granules on both ends. We could not convince ourselves that the parasites invade the red cells. Mayer, too, says that they are found on the surface of the cells. As many as 30 microorganisms may stick to a single erythrocyte. In this stage, there is a great variety in the size and shape of the *Bartonellae*. Further studies will have to show whether the different forms are stages of a cycle or are due to degeneration.

The problem of cultivating the *Bartonella muris* is being investigated by us.

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[1] Mayer, M., Borchardt, W., and Kikuth, W., Beiheft zu Nummer 4 des 31. *Bandes des Archivs fur Schiffs-und Tropenhygiene.* 1927.

[2] Lauda, E., *Wiener Med. Wch.*, 1927, lxxvii, 772.

[3] Nauck, E. G., *Archiv für Schiffs-und Tropenhygiene,* 1927, xxxi, 322.

[4] Haan, E., Lauda, E., and Sorge, G., *Klin. Wch.*, 1927, vi, 2240.

3795

Origin of the Blood Monocyte.*

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The monocytes of the blood were studied in normal rabbits as well as in those in which a monocytosis was produced. This was accomplished by two methods: one was the intravenous injection of lithium carmine, india ink (Higgins') and saccharated iron oxide in various combinations. The other method consisted of infecting the rabbits with *B. monocytogenes* recently isolated by Murray, Webb and Swan.[1] Some of these infected rabbits were also injected with lithium carmine while others were splenectomized. Two of the latter were slowly injected with one quarter of a cc. of

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* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

Higgins' india ink in a branch of the portal vein so that the carbon would accumulate in the endothelial cells of the liver sinusoids.

The blood of the ear veins of all of the rabbits was studied by supravital, dry and wet smears. The animals were killed at various intervals after the appearance of the monocytosis. At necropsy, supravital, dry and wet smears were made of the various organs and vessels. Portions of all of the viscera and of the main vessels, including the thoracic duct, doubly ligated, were fixed in formalin-Zenker, embedded in celloidin, serially sectioned and stained with hematoxylin-eosin-azure II.

Great numbers of monocytes are produced in the rabbits by both of the methods used. These monocytes are characteristic of the classical descriptions of this cell type as studied with May-Grünwald-Giemsa dry smears. They also have the neutral red rosette which Simpson[2] and Sabin, Doan and Cunningham[3] have shown to be typical of the monocyte of the rabbit. In all of the vessels of the body, and particularly in those of the spleen and liver, the monocytes are always accompanied by large numbers of lymphocytes and monocytoid lymphocytes. These latter are so numerous and form such a complete series of transition forms between the lymphocytes and monocytes that the conclusion becomes unavoidable that the monocytes develop by individual transformation of the lymphocytes. The supravital neutral red and janus green stain is the most delicate of the methods used in studying the monocytes. It often shows greatly increased amounts of neutral red in cells which appear to be typical lymphocytes when studied in dry smears and sections of organs.

Particular attention was paid in studying the sections to the relation of the vascular endothelium, reticulum endothelium and fibroblasts to the monocytes. I have found no evidence of any of these fixed cells turning into monocytes. This was clearly shown in the rabbits which were injected with vital dyes either with or without concomitant infection with *B. monocytogenes.* In the carminized animals the monocytes do not contain any of this material while the macrophages contain much of it. The development of the fixed histiocytes into free macrophages can be followed in the sections. Although the monocytes do not contain carmin, they can develop into carmin storing macrophages, (Maximow,[4] Downey and Weidenreich,[5] Jordan,[6] Carrel and Ebeling,[7] Lewis and Lewis[8]).

The evidence of my experiments shows that the monocytes develop from lymphocytes within the blood vessels all over the body and that the sinuses of the spleen are richest in the cells showing this transformation. The numerous mitoses in the lymphocytes in the lymphoid tissue are sufficient to account for the numbers of new cells necessary to produce the monocytosis; there is no such increase in numbers in any of the fixed cells. When the spleen is removed and india ink injected into the portal vein—these procedures eliminating much of the defense of the rabbit against the intravenous injection of the bacteria—the lymphocytes in the lymph nodes and in the bone marrow (hemocytoblasts) develop neutral red rosettes and, in sections, have markedly constructed nuclei. In this case the lymphocytes show evidences of turning into monocytes before they leave the organs in which they are made and before reaching the blood stream.

I am indebted to Dr. Murray for his kindness in sending me several strains of *B. monocytogenes.*

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[1] Murray, E. G. D., Webb, R. A., and Swann, M. B. R., *J. Path. and Bact.*, 1926, xxix, 407.

[2] Simpson, M. E., *J. Med. Res.*, 1922, xliii, 77.

[3] Sabin, F., Doan, C. A., and Cunningham, R. S., *Carnegie Inst. Contrib. to Embryol.*, 1925, xvi, 125.

[4] Maximow, A., *Beitr. z. path. Anat. u. z. allg. Path.*, Suppl. v, 1902; *Klin. Wochnschr.*, 1925, iv, 1486; 1926, v, 2193; *Bindegewebe und Blutbildende Gewebe. Handb. der mikr. Anat. of v. Moellendorf*, J. Springer, Berlin, 1927.

[5] Downey, Hal, and Weidenreich, F., *Arch. f. mikr. Anat.*, 1912, lxxx, 306.

[6] Jordan, H. E., *Am. J. Anat.*, 1925, xxxv, 105.

[7] Carrel, A., and Ebeling, A., *J. Exp. Med.*, 1922, xxxvi, 365.

[8] Lewis, M. R., and Lewis, W. H., *Carnegie Inst. Contrib. to Embryol.*, 1926, xviii, 95.

3796

Preparation of Blood-Free Tissue Proteins.

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It is well known that perfusion and other methods of extraction have hitherto failed to free organs from blood, and for that reason it has been impossible to study the specific proteins in the various tissues or to prepare antisera for them which were not also specific in high dilutions to blood proteins.

In a previous report[1] it was shown that under certain conditions blood-free proteins could be found in the urine which gave precipitin reactions in high dilutions with antisera prepared against liver proteins. It was also suggested[2] that in the early stages of certain nephritides, the liver leaked its proteins into the blood stream and that they were then excreted, as it is well known that the kidneys are permeable to foreign proteins. With these points in view attempts were made to secure larger quantities of this liver protein by animal passage. Solutions containing proteins extracted from dog livers were prepared and freed of non-protein material by dialysis as previously described.[1] This solution contained 0.16% protein. It was injected intravenously into dogs and large amounts of protein were passed in the urine which gave no reactions to blood antisera undiluted, but reacted to liver antisera in dilutions up to 1/100,000.

Injections up to 200 cc. of this solution can be used. Larger amounts tend to produce a blood protein excretion. The proteinuria is slow to begin, reaches its maximum in 12 to 24 hours and subsides after 48 to 69 hours. Reinjection of the same animal will produce a blood proteinuria.

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[1] Loc. cit., 1927, xxv, 102.

[2] *Arch. Int. Med.*, 1927, xi, 548.

3797

Passage of Living Bacteria Through the Intact Intestinal Mucosa.

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A canula was placed in the thoracic duct of dogs under local anesthetic. Lymph was collected for 5 to 10 minutes. The duodenum was exposed through the anterior median line incision in the abdominal wall after local anesthetic had been applied. Forty cc. of solution containing suspended bacteria were injected with proper care and precautions into the lumen of the duodenum. Lymph was collected continuously and divided at 5 minute intervals in separate sterile tubes. The duodenum was immediately replaced and the small incision closed. The experiment was continued for 2 hours. Autopsy was performed to exclude injured duodenal mucosa. Phosphate buffered solutions (pH 8.0) and normal salt solutions were used. Fresh egg white, dog's serum and bile were added to these solutions to make a 10% concentration. *B. prodigiosus* and *B. coli* were suspended in these solutions. The table gives results of experiments with 40 dogs, approximately 5 dogs for each set of experiments.

TABLE I.

Solution injected with bacteria	Average number of bacteria in lymph per cc.	Time of appearance after injection
Alkaline solution and egg white	500 to 1000	1st 5 min. and lasting for 30 min.
Neutral solution and egg white	None	
Alkaline solution	None	
Neutral solution	None	
Alkaline solution and bile	50 to 100	1st 5 min. and lasting for for 20 min.
Neutral solution and bile	3 to 5	During first 30 min.
Alkaline solution and dog's serum	None	
Neutral solution and dog's serum	None	

The duodenum is permeable to living bacteria when the contents are suddenly alkalinized in the presence of a foreign protein; this also takes place when alkalinized bile is placed in the lumen of this part of the intestinal tract. Bile and normal salt solution with bacteria are associated with a slight degree of permeability. This is a preliminary report upon the problem of vaccination by oral ingestion of the antigen.

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Simplified Electrical Method to Distinguish Toxigenic from Non-Toxigenic Diphtheria Bacilli.

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In an earlier paper, Falk, Tonney, White and Jensen[1] reported some details concerning the use of a simple cataphoresis apparatus to distinguish toxigenic from non-toxigenic diphtheria bacilli. There are four shortcomings of the technique: (1) Use of pure cultures; (2) at least 48 hours' incubation; (3) three washings of the bacteria with distilled water; and (4) focussing the microscope on the "stationary level," which have been eliminated by modification of the method.

We now cultivate the suspected culture from the swab on Löffler's blood serum at 37° C. for 12 to 24 hours. The growth is taken off with a sterile, clean wire loop or wooden applicators, and is emulsified in a little distilled water. A little of the emulsion is used for a stained preparation and examined under the microscope. If diphtheria-like organisms are observed, the remainder of the emulsion is used in the capillary tube of the new cataphoresis apparatus.

The principle of the simplified, capillary method was developed by Mooney.[2] The carefully cleaned tube (0.45-0.50 mm. outside and 0.15-0.25 mm. inside) is dipped into the bacterial suspension. It fills by capillarity. It is then laid across the electrodes in the simple chamber which had been previously filled with distilled water. The chamber is merely a rectangular glass dish with a pair of platinum electrodes bent to hold the capillary in a horizontal position. The "cataphoretic velocity" of the bacteria in the middle of the capillary is determined by the method which has already been described in detail.[1] The measurement recorded here, however, is not the true cataphoretic mobility of the bacilli, but the difference between this mobility and the velocity of the endosmotic streaming in the capillary.

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[1] Falk, Tonney, White and Jensen, *Am. J. Pub. Health*, 1927, xvii, 714.

[2] Mooney, *Phys. Rev.*, 1927, xxix, 218.

3799

Chemical Constitution and Germicidal Action.

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In two recent papers Tilley and Schaffer[1, 2] have reported upon the extraordinarily constant relations between chemical constitution and germicidal activity of alcohols, phenols, resorcinols and corresponding intermediate ketones. By correcting the phenol coefficients with the ratio of the molecular weights of the test substance to the molecular weight of phenol, the computed germicidal action for certain organisms is found to vary in a constant ratio with the addition of alkyl groups.

Hence it seemed of interest to determine whether a similar constancy would appear in other chemical series. We have utilized the data reported by Kligler[3] in his studies on the antiseptic properties of certain organic compounds. In a number of series, recomputation of Kligler's data on the germicidal activities of these substances for *Bacterium coli A* (of his series) shows the following ratios:

TABLE I.

	Substance	Molecular Coefficient	Ratio	
A	1. Methyl alcohol	.68		
	2. Ethyl alcohol	1.47	1:2	2.16*
B	3. Aniline	59.4		
	4. Methyl aniline	146.9	3:4	2.5
	5. Di-methyl aniline	389.4	4:5	2.7
C	6. O-toluidine	214.7	3:6	3:6
	7. Methy-O-toluidine	437.0	7:6	2.0
	8. Di-methy-O-toluidine	760.0	8:7	1.7
D	9. P-toluidine	214.7	9:3	3.6
	10. Methy-P-toluidine	437.0	10:9	2.0
	11. Di-methy-P-toluidine	1300.0	11:10	3.0
E	12. Ethyl aniline	283.1	3:12	4.8
	13. Di-ethyl aniline	1710.0	13:12	6.0
F	14. Malachite green	31,040.		
	15. Brilliant green	604,800.	15:14	19.4

*Computed for *Bacterium typhosum*, 2.88. Tilley and Schaffer give 2.2 for this organism.

It is significant to note that the germicidal ratios computed from data prepared by another investigator, working with slightly different technique, give results essentially harmonious with those reported by Tilley and Schaffer. It will be observed that:

(1) In series A, the addition of a methyl group multiplies the germicidal action 2.16-2.88 times. (Tilley and Schaffer obtained ratios of circa 3.1.)

(2) In series B, the addition of a methyl group multiplies the germicidal action of aniline 2.5-2.7 times.

(3) In the toluidine series, Series C and D, both ortho- and para-, the substitution of methyl groups gives approximately the same results.

(4) The substitution of ethyl groups in aniline Series E, multiplies the activity 4.8-6.0 times, or by twice as much.

(5) The replacement of four methyl groups, Series F, in malachite green by corresponding ethyl groups (brilliant green), multiplies the potency nearly 20 times.

This note is presented because it seems important to record a proximate confirmation of work which may significantly clarify the confusion that prevails in the literature on relations between chemical composition and germicidal action.

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[1] Tilley, F. W., and Shaffer, J. M., *J. Bact.*, 1926, xii, 303.

[2] Schaffer, J. M., and Tilley, F. W., *Ibid.*, 1927, xiv, 259.

[3] Kligler, I. J., *J. Exp. Med.*, 1918, xxvii, 463.

3800

Observations on the Cause of Gall-Bladder Contraction and Evacuation.

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In a previous communication,[1] we have shown that purified extracts of the duodenal and jejunal mucosa when injected intravenously cause the gall-bladder to contract and to exert pressure on its contents, and at the same time excite the pancreas to secrete. Since that report, Kloster, Lueth and Ivy have made preparations for us, the use of which indicate that "secretin" does not cause the gall-bladder to contract, but that it is some substance closely associated with "secretin," because it is possible to prepare a solution of "secretin" which is free of the gall-bladder excitant.

In more than 10 experiments in which the cystic duct was clamped and the gall-bladder cannulated under barbital anesthesia, we have observed the gall-bladder to contract when 20 or 30 cc. of N/10 HCl were introduced into the duodenum. The latent period of contraction was in most cases less than 2 minutes, whereas the latent period for the pancreatic response varied from 5 to 10 minutes. We have performed up to the present time, 4 cross-circulation experiments. The cross-circulation experiments were performed as follows: 2 "compatible" dogs were given barbital and placed on the table side by side; the pancreatic duct of each animal was cannulated; the cystic duct of each was clamped and the gall-bladder cannulated and attached to a manometer and recording tambour; the most lateral carotid of each animal was prepared for a blood pressure record, and the remaining or medial carotids were connected with cannulae for cross-circulation. In 3 out of 4 experiments the introduction of 40 cc. of N/10 HCl into the duodenum of the "first" dog caused the gall-bladder of the "second" dog to contract. The gall-bladder of the "second" dog, or the one that did not receive acid in the duodenum, contracted after a period of from 8 to 10 minutes. These experiments demonstrate that the introduction of the acid into the duodenum of the "first" animal caused something to enter the blood of the "first" animal, which passed via the cross-circulation to the "second" animal, causing the gall-bladder of the "second" animal to contract. (The pancreas of the "second" dog, or recipient, was only slightly stimulated in one experiment.)

These observations, if they do not prove, certainly show that a hormone mechanism must be considered, at least, as one of the mechanisms concerned in the normal evacuation of the gall-bladder. We propose the term "cholecystokinin" to designate the active principle which causes the gall-bladder to contract.

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[1] Ivy, A. C. and Oldberg, Eric, PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 113.

3801

Influence of Narcotics on Ciliary Movement of the Gill of the Oyster.

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Ciliary activity is the simplest as well as the most widespread form of specialized contractile activity. The study of its physicochemical conditions is therefore of fundamental interest in relation to the general problem of the conditions of mechanical action in protoplasm. There have been, however, as yet relatively few quantitative studies in this field. One reason for this is the difficulty of exact measurement. In the ciliated epithelium of metazoa the movement is not integrated (as it is in muscular contraction). Each ciliated cell is automatic and independent in its activity, although the direction of effective stroke is typically constant and there is some transmission between neighboring cells. Nervous control is absent or difficult to demonstrate, although it has been observed in certain invertebrates (*e. g.*, veliger of nudibranchs as shown recently by Carter[1]). Control, reversal, and inhibition are best developed in the ciliate Protozoa; electrical sensitivity is also shown most clearly in this group (electrotaxis of Paramoecium, etc.). The cilia of Protozoa are, however, less favorable for studies of narcosis than cilia of a more automatic type, such as those of the ciliated epithelium of metazoa.

In the present study we have chosen cilia having a high degree of persistence, regularity, and automaticity in their movement, namely the cilia of the gill of the oyster, *Ostrea virginiana*. The gill was removed along the gill axis, and then teased into small pieces of about 3 to 5 mm. in width. There are different kinds of cilia in the gill, but the cilia along the ventral margin of the gill were exclusively used. Movement of these cilia can more easily be observed from the side. Five such pieces of gill were placed in each of a series of glass vessels which contained 20 cc. of narcotic solutions of different concentrations in sea water. The vessel, which contained a sufficient free air space for oxygen supply, was covered and sealed with vaseline to prevent escape of narcotic vapor.

After varying periods of exposure, the cilia were observed in the solution, or the gill pieces were returned to sea water, which was renewed several times, and the result was observed on the next day. The temperature was kept at 20° ±1° C. The time of exposure required to narcotize the ciliary movement, and the maximum time of exposure which allows the movement to recover, in different concentrations of each narcotic, were determined. In comparing different compounds, the aim has been to determine as accurately as possible the range of concentration, for each compound, within which the arrest of movement is complete, while at the same time reversible after a period of inactivity lasting at least one hour.

The degree of reversibility varies greatly with different compounds. In general, the recovery of activity on return to normal sea water becomes less complete as the duration of the narcosis increases; *i. e.*, the narcotic or reversible action passes by degrees into a toxic or irreversible action at a rate increasing with concentration. The rate of toxic action, at the optimum narcotizing concentration, varies with the different compounds, some compounds such as

TABLE I.

Compounds	Narcotic Range, M.	Optimum Concentration, M.	Q
Alcohols:			
Methyl	3 -5	4	2
Ethyl	2±	2	3.3
n-Propyl	0.5 -0.75	0.6	3.2
n-Butyl	0.187-0.25	0.187	3.0
i-Amyl	0.062-0.093	0.062	(3.1 ?)
n-Heptyl	(0.0062 ?)	(0.0062 ?)	(1.9 ?)
Capryl	(0.0033 ?)	(0.0033 ?)	
Urethanes:			
Ethyl	0.5±	0.5	2.58
n-Butyl	0.08 -0.075	0.075	3.0
i-Amyl	0.025±	0.025	
Phenyl	*ca.* 0.004	*ca.* 0.004	
Nitriles:			
Aceto-	0.5 -1.0	1.0	2.0
Propio-	0.5 -0.75	0.5	2.0
n-Butyro-	0.2 -0.25	0.25	
Ketones:			
Methyl methyl	1.5 -2.0	1.5	
Methyl propyl	0.175-0.20	0.175	2.9
Methyl phenyl	0.025 ?	0.025 ?	
Chloral hydrate	0.1 -0.025	0.025	
Chloretone	0.01 -0.015	0.01	

chloral hydrate, aceto-nitrile, and chloretone being relatively non-toxic, while some others, *e. g.*, ketones, ethyl urethane, rapidly cause irreversible changes in the tissue. These differences appear arbitrary, just as do the analogous differences in the action of different anesthetizing compounds on higher animals.

Physiologically equivalent concentrations of the different compounds, *i. e.*, those in which there is cessation of movement lasting one hour or more and admitting of more or less complete revival, are given in the table.

In general the following features appear clearly from these experiments. In homologous series the optimum narcotizing concentrations decrease as the number of C-atoms in the chain increases, the numerical factor of increase, Q, on passing from one member to the next being typically between 2 and 3; sometimes larger than 3, *e. g.*, 3.3 with ethyl and propyl alcohols.

The rate of narcotic action also varies in the different members of a series, being in general slower with the higher members of the series. The rate of recovery is also slower in the higher members. This difference is probably to be related to the rate of diffusion. The optimum concentration is more sharply defined in the higher members of the series; *i. e.*, a slight increase above the optimum concentration in such compounds may render the effect irreversible.

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[1] Carter, G. S., *Brit. J. Exp. Biol.*, 1926, iv, 1.

Western New York Branch.

Clifton Springs Sanatarium, December 12, 1927.

3802

Effect of Kidney on Blood Regeneration in Pernicious Anemia.

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The experiments of Whipple and Robscheit-Robbins[1] have given rise to an extensive knowledge of the effects of various articles of diet on the regeneration of blood in dogs rendered chronically anemic by repeated bleeding. The most important application of this fundamental work to the therapy of anemia was made by Minot and Murphy,[2] who reported extraordinarily good results from the administration of liver in pernicious anemia. These results have been abundantly confirmed. The use of liver in the diet has become so general that it is frequently difficult for patients to obtain it, so much so that it is necessary to investigate the therapeutic effects of other foods which Whipple and Robscheit-Robbins have found to be effective, particularly kidney and apricots, both of which caused active regeneration in the anemic dogs.

In this paper are reported observations on the course of blood regeneration in two cases of pernicious anemia who were fed diets containing 250 gm. of kidney daily. The diets contained no red muscle, no foods other than kidney found to be of high value in blood regeneration by Whipple and Robscheit-Robbins, except small amounts of apricots, prunes, and other fruits, given as desserts.

Patient No. 11028, a man aged 68 years, was admitted to the Strong Memorial Hospital for symptoms of weakness, anorexia, sore tongue, loss of weight, and paraesthesias in hands and feet, of 7 months duration. On examination the principal findings were, yellowish pallor, red tongue with smooth borders, moderate smooth sclerosis of palpable vessels. Vibratory sense unimpaired. Reflexes all in order. There was achylia gastrica, urobilinuria, and a slight increase in blood plasma pigment—Van den Bergh test 1.8 units. The hemoglobin was 45% Sahli (100% = 14.5 gm. Hgb.

per 100 cc.). Red corpuscles 1.42 millions per cu. mm. Leucocytes 4,100. Blood smear showed anisocytosis, poikilocytosis, some diffuse and punctate basophilia. Reticulocytes 1.4%.

Diagnosis: Pernicious anemia. The course of events after administration of the kidney diet, which was taken well for 17 days, is shown in Fig. 1. In 17 days the red count rose from 1.4 to 2.6 millions, the hemoglobin from 45 to 74%. The percentage of reticulocytes, which was slightly elevated at the start, varies between 2 and 5% between the 7th and 16th days. On the 17th day liver was given instead of kidney and on the 6th day thereafter the hemoglobin had risen from 74 to 80%, and the red count from 2.6 to 2.8 millions. The leucocytes count rose from 4100 to 7200. All symptoms were much improved.

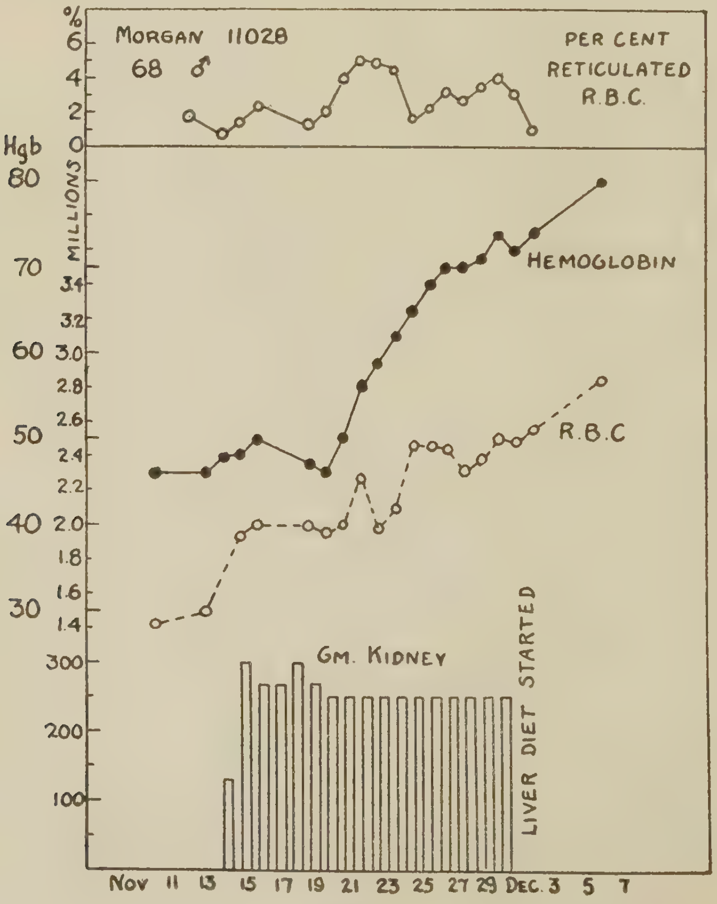

FIG. 1.

Patient No. 10861, a woman of 71 years, was admitted to the Strong Memorial Hospital complaining of vague gastro-intestinal symptoms of 2 years duration, numbness of hands and feet of 8 months duration, soreness of tongue, 4 to 5 months duration, and great weakness. Examination revealed pallor, emaciation, senile keratosis of skin; arcus senilis, retinal arteriolar-sclerosis, early cataracts; atrophy of marginal papillae of tongue; systolic bruits over both apex and base of heart, peripheral arteriosclerosis, B. P. 160 mm. systolic, 70 mm. diastolic; palpable liver; moderate pitting edema of extremities; neurological—considerable ataxia of gait and station and loss of postural sense, impairment of vibratory sense; ankle jerks absent, abdominal reflexes not obtained, plantar reflexes of flexor type. The blood on admission: Hemoglobin 65%

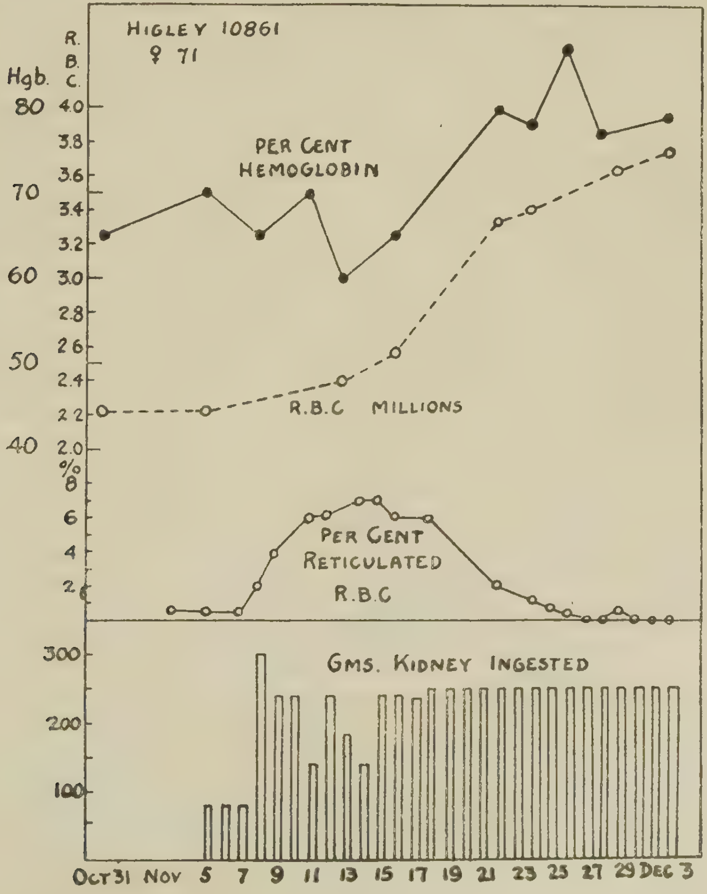

FIG. 2.

Sahli (100 = 14.5 gm. per 100 cc.). R. B. C. 2.24 million, W. B. C. 5,800. Smear showed anisocytosis with many macrocytes, considerable poikilocytosis, no obvious reduction of platelets. The plasma bilirubin was not increased above normal. Reticulocytes 0.7%. The gastric contents showed no free hydrochloric acid.

Diagnosis: Pernicious anemia, with subacute combined degeneration of spinal cord and moderate anemia, with cord symptoms progressing more rapidly than the anemia. The course under treatment with kidney diet is best shown in Fig. 2. The kidney was given for a period of 27 days, during which time the red cell count rose from 2.2 to 3.8 millions, the hemoglobin from 65 to 79%. The leucocytes increased from 5,800 to 10,000 per cu. mm. The percentage of reticulocytes began to increase on the 3rd day and reached a maximum of 7% on the 7th and 8th days, reaching normal again of the 17th day of the diet. As the reticulated cell count fell the total red cell count rose. During the course of observation the spinal cord symptoms grew worse, if anything, though the general condition of the patient improved.

Two patients with pernicious anemia had remissions induced by giving a diet with 250 gm. of kidney daily. The relative values of kidney and liver feeding can be determined only on comparison of a long series of cases.

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[1] Whipple, G. H., and Robscheit-Robbins, F. S., *Am. J. Physiol.*, 1925, lxxii, 395, 408, 419, 431; 1927, lxxix, 260, 271.

[2] Minot, Geo., and Murphy, Wm. P., *J. Am. Med. Assn.*, 1926, lxxxvii, 47; *Bost. Med. and Surg. J.*, 1926, cxcv, 410.

3803

Influence of Epinephrine on Carbohydrate Metabolism of Fasting Rats.

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A brief report[1] has been made concerning the influence of epinephrine injections on the utilization of ingested glucose in rats, and of other experiments which dealt with the metabolic changes brought about by epinephrine in post-absorptive rats, rich in glycogen. In both the sugar fed and the post-absorptive rat, there was a diminution in muscle glycogen, which indicates that epinephrine has also a point of attack in the peripheral tissues. The present experiments were made on rats fasted previously for 24 hours. After such a fasting period, the glycogen content of the rats is very constant, a point which has been established in previous work. It is also important to mention that the glycogen of the rat undergoes only a slight diminution, between the 24th and the 48th hour of fasting. This is shown in the following table, which has been calculated from previous experiments:

Glycogen Content (in mg. per 100 gm. rat)

	liver	body	total	
24 hour fasting	7	136	143	(average of 16)
48 hour fasting	10	111	121	(average of 21)
Difference	+3	—25	—22	

The present control series, which is included in the above average, showed the following values:

glycogen in liver (mg.)	3.0,	4.2,	9.9,	7.1.
glycogen in body (mg.)	123.0,	129.8,	144.1,	114.9.

In the experimental rats the average R. Q. of a 3 hour metabolism fore period, was 0.715. After the subcutaneous injection of 0.02 mg. of epinephrine, there was no perceptible rise in the R. Q., the average for a 3 hour period being 0.715. The O_2 consumption rose markedly in each experiment, with an average increase of 18.1%. The rats were killed 3 hours after the injection (after the same fasting period as the control rats) and the glycogen was determined in the liver and the rest of the body. The following values, calculated per 100 gm. of body weight were obtained:

glycogen in liver (mg.)	28.4,	50.2,	45.9,	43.1,	32.6,	51.8.
glycogen in body (mg.)	58.4,	81.9,	69.5,	76.5,	62.8,	77.2.

It will be seen from a comparison of these values with the values obtained on merely fasted controls, that there is a marked increase in the liver glycogen in every experiment. At the same time, the glycogen in the body is found to have decreased, on an average, a little more than the liver glycogen has increased (—57 mg. against +36 mg.).

An increase in liver glycogen, after epinephrine injections into fasting rabbits, has been observed by Pollak[2] and by Kuriyama.[3] In the latter author's work are included some determinations of muscle glycogen. On an average, the muscle glycogen in his injected rabbits is lower than in the controls.

The possibility suggests itself that the glycogen mobilized in the peripheral tissues (mainly muscles), has been deposited as liver glycogen. If the disappearing muscle glycogen (57 mg.) were oxidized, the R. Q. would be between 0.73 and 0.74. It is now definitely known, especially from Mann's[4] work on dehepatized dogs, that muscle glycogen does not contribute blood sugar after epinephrine injections or under any other conditions. When muscle glycogen is split, the presence of the glycolytic ferment causes an immediate change of the split products into lactic acid (Lohmann[5]). The course of events, in 24 hour fasting rats, would then be that epinephrine mobilizes muscle glycogen, and that lactic acid enters the blood stream and is carried to the liver, where it is deposited as glycogen. (In post-absorptive rats, which are rich in glycogen, the muscle glycogen mobilized by epinephrine is almost completely oxidized.)

Before considering this assumption further, it was necessary to determine whether lactic acid is able to form liver glycogen. 3.5 cc. of a 6% solution of d-lactic acid* (unneutralized) was fed by stomach tube to 24 hour fasting rats. The animals showed neither toxic symptoms, nor lesions of the stomach and intestines. They were killed after 3 hours and the liver glycogen and absorption was determined. The following values (calculated per 100 gm. of body weight):
glycogen in liver (mg.) 57.2, 50.8, 32.1, 24.0, 59.9, 34.3, reveal that liver glycogen is deposited during d-lactic acid absorption. One possible obstacle in the explanation here proposed has therefore been eliminated. An older observation on rabbits[6] gains special significance in the light of the present work. It was found that epinephrine injections caused a strong increase in blood lactic acid. An increase has also been found, to a lesser extent, in cats and in the rats used for the present investigation.

Though the explanation here proposed seems well supported, the possibility cannot be excluded that the liver glycogen accumulating during epinephrine action is derived from non-carbohydrate (protein) sources. If this were the case, one would have to assume that the glycogen disappearing in the rest of the body, is oxidized. The combination of the 2 processes (anabolic and catabolic) would also result in the R. Q. actually observed.

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* For the preparation of d-lactic acid, we are indebted to Dr. Peterson of the College of Agriculture, University of Wisconsin.

[1] Cori, C. F., and Cori, G. T., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 66.

[2] Pollak, L., *Arch. exp. Path. u. Pharm.*, 1909, lxi, 166.

[3] Kuriyama, S., *J. Biol. Chem.*, 1918, xxxiv, 269.

[4] Mann, F. C., and Magath, T. B., *Am. J. Physiol.*, 1925, lxxii, 629.
[5] Lohmann, K., *Biochem. Z.*, 1926, clxxviii, 444.
[6] Cori, C. F., *J. Biol. Chem.*, 1918, xxxiv, 269.

3804

Urea and Creatinine Concentration in the Blood; a Statistical Study.

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The relationship between the accumulation of urea and creatinine in the blood has held considerable interest since Myers[1] reported evidence which tended to show that in nephritis urea increased in the blood before creatinine did. It seemed probable that data obtained in a clinic where most of the patients were undergoing treatment for chronic diseases might show some relationships between these compounds which would be of value. Results obtained over a period of eight years were therefore tabulated and studied. All cases where analyses of both compounds in the same sample of blood were available were utilized. Such studies were not made on all patients, but blood was obtained from most of those in whom the presence of renal insufficiency was suspected. During the greater part of the period creatinine determinations were done on all specimens where a urea nitrogen concentration greater than 20 mg. per 100 cc. was found, but were generally omitted when the concentration was lower than this figure. During the earlier part of the period both substances were determined in most specimens submitted. The method of Folin and Wu[2] was used for the creatinine determination. During the last two years an unpublished method of Folin and Sumner was used for determining the urea nitrogen; before that time the technique recommended by Van Slyke and Cullen[3] was followed.

The average results are given in the table. The two halves of the table are alike except that in the right hand portion normal and slightly increased values are given at smaller intervals than in the left hand part. It is evident that, except when the urea nitrogen concentrations were low, the urea and creatinine values tended to vary together. Holbrook and Haskins[4] have recently reported similar findings in a series of selected cases. When the urea nitrogen concentration was slightly increased the ratio between creatinine and urea was approximately the same as when the urea was mark-

Urea Nitrogen	No.	Creatinine	Ratio	Urea Nitrogen	No.	Creatinine	Ratio
0.0 to 4.9	3	1.33	0.333	0.0 to 8.0	25	1.44	0.213
5.0 to 9.9	60	1.43	0.172	8.0 to 8.9	16	1.36	0.163
10.0 to 14.9	239	1.51	0.119	9.0 to 9.9	22	1.45	0.153
15.0 to 19.9	190	1.61	0.095	10.0 to 10.9	30	1.44	0.136
20.0 to 24.9	128	1.82	0.083	11.0 to 11.9	56	1.51	0.131
25.0 to 29.9	86	2.03	0.075	12.0 to 12.9	50	1.54	0.123
30.0 to 34.9	49	2.51	0.075	13.0 to 13.9	42	1.50	0.112
35.0 to 39.9	29	2.81	0.076	14.0 to 14.9	61	1.53	0.107
40.0 to 44.9	22	2.92	0.069	15.0 to 15.9	56	1.54	0.100
45.0 to 49.9	22	3.37	0.071	16.0 to 16.9	64	1.64	0.100
50.0 to 54.9	19	2.68	0.052	17.0 to 17.9	22	1.66	0.095
55.0 to 59.9	12	4.42	0.077	18.0 to 18.9	31	1.64	0.089
60.0 to 64.9	14	5.55	0.089	19.0 to 19.9	17	1.63	0.084
65.0 to 69.9	14	5.34	0.081	20.0 to 20.9	30	1.66	0.081
70.0 to 74.9	6	4.32	0.059	21.0 to 21.9	32	1.84	0.086
75.0 to 79.9	3	6.83	0.088	22.0 to 22.9	24	1.95	0.088
80.0 to 84.9	4	6.28	0.075	23.0 to 23.9	26	1.83	0.079
85.0 to 89.9	2	4.15	0.047	24.0 to 24.9	16	1.86	0.076
90.0 to 94.9	3	8.13	0.089	25.0 to 25.9	28	1.91	0.075
95.0 to 99.9	2	8.35	0.085	26.0 to 26.9	13	2.04	0.077
100.0 to 104.9	2	8.95	0.088	27.0 to 27.9	17	2.01	0.073
105.0 to 109.9	5	10.14	0.094	28.0 to 28.9	19	2.08	0.073
110.0 to 114.9	4	7.78	0.070	29.0 to 29.9	9	2.40	0.082
115.0 to 119.9	3	10.03	0.084	30.0 to 34.9	49	2.51	0.075
120.0 to 124.9	6	9.35	0.077	35.0 to 39.9	29	2.81	0.076
125.0 to 129.9	1	13.30	0.103	40.0 to 49.9	44	3.15	0.070
135.0 to 139.9	3	7.30	0.054	50.0 to 59.9	31	3.35	0.062
140.0 to 144.9	1	14.50	0.101	60.0 to 69.9	28	5.44	0.085
150.0 to 154.9	2	9.35	0.062	70.0 to 79.9	9	5.16	0.069
155.0 to 159.9	1	16.00	0.101	80.0 to 89.9	6	5.57	0.066
160.0 to 164.9	1	15.00	0.092	90.0 to 99.9	5	8.22	0.087
170.0 to 174.9	2	9.75	0.057	100.0 to 109.9	7	9.66	0.090
175.0 to 179.9	1	10.30	0.059	110.0 to 119.9	7	8.74	0.076
180.0 to 184.9	1	13.30	0.073	120.0 to 129.9	7	9.91	0.081
200.0 to 204.9	2	9.90	0.050	130.0 to 149.9	4	9.10	0.067
215.0 to 219.9	1	4.60	0.021	150.0 to 174.9	6	11.53	0.071
235.0 to 240.0	2	13.00	0.054	175.0 to 199.9	2	11.80	0.066
290.0 to 294.9	1	18.40	0.063	200.0 to 299.9	6	11.47	0.050

Urea nitrogen and creatinine determinations are expressed as mg. per 100 cc. blood.
The ratio was obtained by dividing the creatinine by the urea nitrogen.

edly abnormal. There is no evidence that there was a group of patients with slight increases in urea nitrogen but with normal creatinine values, as would be expected if the kidney was more permeable to creatinine than to urea.[5] A study of the distribution of individual urea and creatinine values confirmed the impression given by the averages.

Cases where the results of more than one study upon the same patient were available were also analyzed. There were 230 successive determinations of this sort. When both the urea and creatinine increased the urea tended to increase faster than did the creatinine, but when both decreased the urea also tended to decrease more rapidly. Almost all of the relationships observed could be explained on the basis of variations in the urea concentration similar to those known to take place in intestinal obstruction. It seems more logical to attribute these variations to changes in the rate of formation rather than of elimination of urea. Such variations in the rate of formation would, of course, cause greater changes in the blood urea concentration when kidney function is impaired than when it is normal. Such a hypothesis would explain better the very high blood urea nitrogen values accompanied by normal creatinine concentrations than does the assumption of a different permeability of the kidney for these two compounds. Part of the figures could be explained by introducing a time factor into the conception. The urea may accumulate earlier in the blood than does creatinine, and the creatinine later rise fairly rapidly to a comparable level. Such an explanation can be applied only with difficulty to much of the data obtained in successive determinations on the same patient; for instance, to the facts that there was a more rapid fall of urea than of creatinine when both were decreasing, and that where the urea was constant there were as many cases which showed a drop as showed a rise in creatinine (although the average per cent rise was greater than the average fall). The evidence in favor of variations in the rate of creatinine formation was at least as slight as was that in favor of the proposition just stated. It seems best not to press explanations which apply only to a very small proportion of the determinations analyzed, but rather to conclude that, in our series, composed almost wholly of chronic cases, with acute nephritis very meagerly represented, urea and creatinine concentrations in the blood paralleled each other; that there was not infrequently evidence of rather sudden changes in urea concentration probably associated with variations in the rate of urea formation; and that there was a small number of cases where some additional factor or factors seemed to be affecting the results.

[1] Myers, V. C., *J. Biol. Chem.*, 1919, xxxviii, 239; *Am. J. Med. Sci.*, 1919, clvii, 674.

[2] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii, 91.

[3] Van Slyke, D. D., and Cullen, G. E., *J. Am. Med. Assn.*, 1914, lxii, 1558.

[4] Holbrook, P. H., and Haskins, D. H., *J. Lab. Clin. Med.*, 1926, xii, 10.

[5] Hubbard, R. S., *Clifton Med. Bul.*, 1923, ix, 10.

3805

Calcium Content of Maternal and Foetal Blood Serum Following Injection of Parathyroid Extract in Foetuses in Utero.

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In considering the changes in the maternal organism during pregnancy many observers from the time of Rokitansky have noted alterations in the teeth and skeletal tissues.[1] In this connection, Seitz[2] has pointed out that "of all the mineral substances, calcium passes in the greatest amount from the mother to the foetus" during gestation. It has been found also that about the time of very active development of bone in the foetus, which probably necessitates the mobilization of a considerable supply of calcium in the foetal body, the parathyroid glands of the embryo are already differentiated morphologically.[3] Of immediate interest, however, are the recent investigations of Collip,[4] as a result of which there is available an extract of the parathyroid glands which exerts a powerful influence on the mobilization of calcium in the blood. By means of this highly concentrated parathyroid substance, the old problem of the mechanism which is involved in the exchange of calcium from mother to foetus is reopened to experimental inquiry from a new angle. The possibility of the escape of active parathyroid substance from the foetus to the mother across the placental boundary, can be tested by direct observation.

In the present experiments, parathyroid extract was injected into foetuses *in utero*. Observations were made upon the calcium content of the maternal blood serum at intervals of about 4 hours, in order to detect any changes in the calcium level of the maternal blood, such as might be produced by the escape of parathyroid substance into the maternal circulation. About 17 to 22 hours after the injection of the foetuses, the uterus was opened and blood samples for calcium determinations were taken from all of the foetuses. The

TABLE I—Calcium Content of Maternal and Foetal Blood Serum following the Injection of Parathyroid Extract in Foetuses *in Utero.*

					Serum Calcium (mg. per 100 cc.)					Control		
					Foetal		Maternal				Serum Calcium	
Dog No.	Stage of Pregnancy	Weight (kilos)	Units of Parathyroid Extract Inj.	Number Foetuses Injected	Injected Foetuses	Non-injected Foetuses	Before injection of Foetuses	Highest level during experiment	Duration of Experiment (hours)	Dose (units)	Before injection	After injection
1	Near term	12.1	120	4	18.2 14.8 16.2 18.4	12.6 12.1 12.5	11.1	11.3	21	70	10.7	15.4
2	Very near term	24.3	100	3	13.5	13.8 13.3 13.2 13.5 13.8 13.4 14.0 13.1 13.4	11.2	10.3	21½	100	10.1	14.8
3	Near term	20.4	100	3	14.0 15.8 16.0	12.0 13.0 12.3 12.2 12.8 12.6 12.2	11.2	11.0	18	80	11.2	13.2
4	Near term	16.7	100	3	17.2 18.6 17.3	13.8 13.6 13.6	11.9	12.0	17	60	11.0	15.1
5	About 8 weeks	16.0	100	5	15.8 19.2 19 8		9.8	11.1	22	(See	above)	

findings in the case of each dog are summarized in Table I, and the procedure in a typical experiment is outlined in the graph (Fig. 1).

In these experiments in which parathyroid extract (Collip) was injected into foetuses in the dog, there is no evidence that the active substance reached the maternal circulation, as indicated by the calcium content of the maternal blood serum. The foetal blood serum obtained at the end of each experiment was found in the non-injected foetuses to be about 1 to 3 mg. higher in calcium than the maternal serum. In foetuses which had been injected previously with parathyroid substance, the calcium content of the serum was more than 3 mg. higher than in the maternal serum. The present

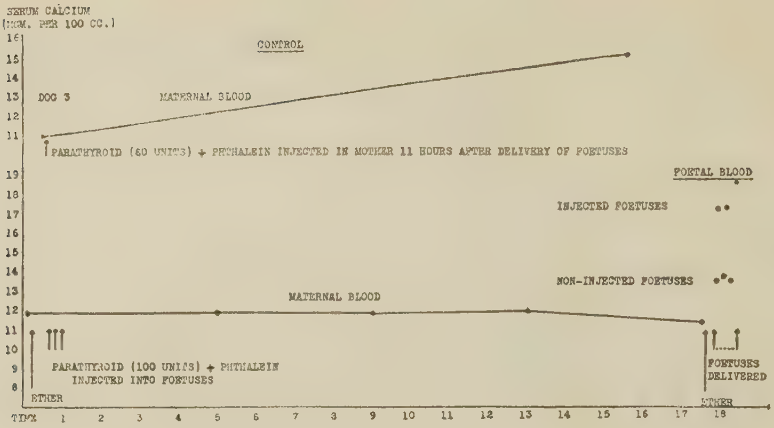

FIG. 1.

findings in the case of parathyroid extract are in agreement with previous observations which have been made upon the effect on the mother of the injection of insulin, adrenalin, and pituitrin in rabbit and guinea pig foetuses, *in utero*. No evidence has been obtained that these endocrine substances pass from foetus to mother across the placental boundary.[5]

The parathyroid extract (Collip) or "Para-thor-mone" used in the experiments was donated by Eli Lilly & Co.

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[1] Williams, J. W., *Text-book of Obstetrics*, 1924, 196.

[2] Seitz, L., *Handbuch der normalen und pathologischen Physiologie*, 1926, xiv, 485.

[3] Fischer-Wasels, B., and Berberich, J., *Hirsch's Handbuch der Inneren Sekretion*, 1927, i, 432.

[4] Collip, J. B., *The Parathyroid Glands, Medicine*, 1926, v, 1.

[5] Snyder, F. F., and Hoskins, F. M., *Anat. Rec.*, 1927, xxxv, 23.

3806

Cephalically Situated Respiratory Mechanism in the Brain Stem of the Cat.

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The origin and course of the reticulospinal tracts in the cat, recently described by Papez[1] suggested the possibility of their being respiratory tracts. It was thought that this problem could best be approached by localizing the respiratory mechanism in this region of the brain stem. The material reported in this paper summarizes the results obtained thus far. The animals were given 1 gm. of urethane per kilo of body weight. Both carotids were tied at the time of cannulation of the trachea. Ether was used during surgical procedure. As the animal came from under ether, hyperpnea usually resulted spontaneously.[2] Hyperpnea was hastened by heating the preparation, or as in most cases, merely by stimulating the central sciatic stump with moderately strong induction shocks. This "rapid respiration mechanism" could be "shot" in the following ways: 1—exposing the corpora quadrigemina; 2—sectioning the brain stem through, or caudal to, the superior colliculi. However, the superior colliculi can be exposed without interfering at all with the mechanism. It was also found that a complete section of the brain stem cephalic to the corpora quadrigemina did not alter the respiratory rate.[3]

A further study of this mechanism has been made possible by a method which exposes the floor of the fourth ventricle without interfering with respiration. By this method it has been found that a longitudinal lesion passing ventral to the corpora quadrigemina and lateral to the mid-line will *slow* respiration. But, heating the animal after this effect is produced, will *increase* the rate of respiration. It was noted, however, that this latter increase was not nearly as marked as the increase in respiration which was caused by heating the animal previous to placing the lesion.

The fact that the mechanism can be partially "shot" by lesions affecting one side of the stem only, is demonstrated very forcibly by placing a transverse lesion on one side involving the lateral region of the stem. Such a lesion can be placed at any level between the inferior colliculi and the facial nuclei. After such a lesion, the respiratory rate is slowed. Each respiration is deeper and both the expiratory and inspiratory phases are lengthened. However, between the latter respiratory acts, short rapid excursions are superimposed upon the inspiratory phase of the larger and slower excursions. A corresponding lesion on the opposite side eliminates the short rapid excursions entirely. The respiratory mechanism in an animal having two such lesions is so affected as to cause no increase in respiration when the animal is heated. Extensive lesions, transverse or longitudinal, through the medial reticular formation at any place within the above levels do not interfere with the respiratory rate.

The above results suggest, in general, a center which is concerned with the production of rapid respiration located somewhere in the region of the inferior colliculi, possibly in the cephalic pons.[4] The tracts concerned, probably afferent,[5] pass through the medulla and pons in a lateral position.

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[1] Papez, J. W., *J. Comp. Neur.*, 1926, xli, 365.

[2] Henderson, Yandell, *Am. J. Physiol.*, 1910, xxv, 310.

[3] Trevan, J. W., *J. Physiol.*, 1916, l, 43.

[4] Lumsden, T., *J. Physiol.*, 1923, lvii, 153.

[5] Coombs and Pike, *Am. J. Physiol.*, 1918, xlv, 569.

3807

Experimental Production of Gastric Ulcer.

P. T. McILROY. (Introduced by Herbert U. Williams.)

From the School of Medicine, University of Buffalo.

My interest in the experimental production of gastric ulcer was aroused by the finding of two well defined ulcers in the stomach of a cat which had received injections of histamine for another investigation. The cat is a suitable animal to use for the experimental production of gastric ulcers. It exists on a mixed diet comparable to that of man and develops ulcers fairly readily. The above observation led me to examine carefully the stomachs of all cats treated by histamine. Of 4 cats examined 1 showed a spontaneous ulcer on the lesser curvature 1.5 cm. from the pylorus.

There are 2 problems in the pathology of gastric ulcer, first the origin of the initial lesion, and second, the cause of the chronic character. It is a well established fact that histamine when injected subcutaneously increases the flow of gastric secretion and especially the hydrochloric acid. The acid content of the gastric juice has been regarded by some for many years as a potent factor in the causation of chronic gastric ulcer. Ulcers of the peptic type occur in 4 situations: (1) stomach; (2) duodenum; (3) stoma of gastro-jejunostomy; (4) lower end of oesophagus. These areas are more or less continuously in contact with gastric secretion. Gastric ulcers have been known to follow injury caused by the passing of a stomach tube.

The following experiments have been carried out in order to ascertain whether or not trauma associated with hyperacidity might be causative factors in the production of gastric ulcers. I traumatized the gastric mucosa in the following manner: laparotomy was performed and the stomach exposed. The serosa was incised and dissected back exposing 5 mm. of the subserous tissue. A piece of brass wire 3 mm. in diameter heated to a dull red was plunged through the remaining stomach layers. The stomach wound was closed by 2 layers of purse-string sutures and the laparotomy wound closed in the usual way. Twenty-four animals have been so treated. Seventeen were used as controls while the remaining 7 were treated by injections of histamine (ergamine acid phosphate B & W) in amounts varying from 10 to 20 mg. per dose. Injections were begun 2 days after operation and continued every alternate day thereafter until the animal was chloroformed.

In order to determine the approximate length of time required for the mucosa in different areas of the stomach to heal, 8 received cardiac trauma, 5 pyloric and 3 both cardiac and pyloric. In all of the above the cardiac lesions had healed completely in 14 days while those in the pylorus required 17 days at least. In the animals which received both cardiac and pyloric injuries the latter were not healed in any case, proving conclusively that it requires a longer interval for the healing of pyloric lesions. The pylorus was therefore chosen as being the most suitable location for the further carrying out of this experiment.

Pyloric lesions were therefore produced in the remaining 7 animals and in addition each received subcutaneous injections of histamine with the following results:

Cat	*Length of time treated*	*Result*
1	8 days	Extension of original lesion. No attempt at healing
2	12 days	Extension of original healing. Perforation
3	15 days	Extension of original lesion
4	15 days	Extension of original lesion
5	15 days	Extension of original lesion
6	11 days	Extension of original lesion
7	11 days	No extension of original lesion. Some attempt at healing. Three small ulcers in mucosa and pylorus

Although in none of the above may it be claimed that a chronic peptic ulcer has been produced, I believe that histamine in some as yet undetermined manner led, undoubtedly, to the extension of the lesions produced. It appears probable, however, that the extension of the lesion was due to the increase of hydrochloric acid induced by the action of the histamine. Further experimentation may clarify this point.

I wish to thank Dr. H. U. Williams for invaluable suggestions relative to the carrying out of this work.

Pacific Coast Branch.

Stanford University School of Medicine, December 14, 1927.

3808

Further Experiments on the Fate of Taurine in Dogs.

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Experiments which were carried out in this laboratory by Schmidt and his co-workers[1, 2] showed that when taurine is ingested by man there is no marked increase in urinary sulfates. The α-amino nitrogen determinations in the urine indicated that contrary to the claims of Salkowski, taurine is excreted free and not as taurocarbamic acid. Schmidt and Clark[3] were able to show that the fate of taurine in dogs is the same as in man.

The experiments described below were undertaken with the view of isolating taurine from the urine of dogs which had been fed this substance. For the isolation of taurine we used a modification of the method described by Bergell.[4] The Bergell technique in our hands did not yield positive results. The procedure was as follows: One gm. taurine was dissolved in 100 cc. of urine. To this solution were added 2.5 gm. β-Naphthalene-sulfochloride, in 15 cc. ether, and 9 cc. N-NaOH. After shaking for 2 hours, 1 gm. of the chloride in 10 cc. ether, and 10 cc. N-NaOH were added. The shaking was continued for 8 more hours, care being taken that the reaction remained alkaline throughout the experiment. After completion of the reaction, the aqueous layer was separated and acidified with dilute HCl. A fine sediment separated out. The mixture was placed in the ice-chest over night. The next morning, it was centrifuged and just enough sodium chloride added, until the Na-salt of β-Naphthalene- sulfotaurine began to form. After standing in the ice-chest for 24 hours, the precipitate was filtered, dried and weighed. The substance was purified by recrystallization from 85% alcohol. By this method we were able to recover 0.8 gm. from 1.0 gm. taurine added to 100 cc. of urine.

The following results were obtained from animal experiments: Dog 1 received 3.0 gm. taurine in 100 cc. of water by stomach tube. From the urine of the first and second days a total amount of 5.10 gm. Na-β-Naphthalenesulfotaurine, equivalent to 2.2 gm. taurine, were recovered. Recovery 73%. Dog 2 was given 4.0 gm. taurine in 100 cc. of water by stomach tube. From the urine 5.90 gm. Na-β-Naphthalenesulfotaurine, equivalent to 2.6 gm. taurine, were obtained. Recovery 65%.

These results confirm the previous work carried out in this laboratory, and show within the errors of experimentation that taurine when fed to dogs is excreted unchanged, and not in the form of taurocarbamic acid.

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[1] Schmidt, C. L. A., von Adelung, E., and Watson, T., *J. Biol. Chem.*, 1918, xxxiii, 501.

[2] Schmidt, C. L. A., and Allen, E. G., *J. Biol. Chem.*, 1920, xlii, 55.

[3] Schmidt, C. L. A., and Clark, G. W., *J. Biol. Chem.*, 1922, liii, 193.

[4] Bergell, P., *Z. physiol. chem.*, 1916, xcvii, 260.

3809

Osmotic Pressure Measurements on Proteins in Urea Solutions.

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In the development of the theory of solutions, it has often happened that the use of other solvents than water has led to results that are entirely obscured in aqueous solutions.[1] It seems desirable on this account to study the physical chemical properties of proteins in such other solvents as are capable of dissolving them in appreciable quantities. A few such pure and mixed solvents that might be employed are anhydrous formic acid, liquid phenol, alcohol-water mixtures and urea-water mixtures.

As a start on this general problem, measurements of the osmotic pressure of proteins in urea solutions were undertaken. The 2 immediate objectives were to determine if changes in the state of aggregation of proteins could be detected, and, to develop a method for the determination of the molecular weights of proteins that are not soluble in water in the absence of electrolytes. Up to the present, the only means of fairly accurately determining the molecular weights of proteins (colloids in general) that have been developed are by osmotic pressure[2] determinations and by the ultracentrifuge[3] method of Svedberg. In both of these methods pure isoelectric solutions of proteins are required to obtain correct results. Only relatively few proteins are soluble in water in the isoelectric condition. Urea solutions have a very powerful solvent action on most proteins. If the protein is a pure, electrolyte free sample, the resulting solutions show only a very slight conductivity indicating that the dissolved protein is unionized. This makes it possible to make the osmotic pressure measurements with the elimination of the Donnan membrane equilibrium as an interfering factor.

TABLE I.
Osmotic Pressure of Casein in 40% urea solution.

Concentration of Casein (gm. per 100 cc.)	Casein gm. per 100 gm. solvent	Observed Osmotic Pressure. cm. of solution	Capillary Correction cm.	Osmotic Pressure. Height in cm. of H_2O
Series 1.				
1.01	0.93	12.5	0.52	13.2
1.95	1.80	26.6	0.43	29.0
2.68	2.48	39.0	0.43	42.7
3.66	3.42	51.0	0.43	56.1
Series 2.				
0.95	0.87	11.75	0.43	12.45
3.63	3.40	53.2	0.43	58.5

In Table I are given some measurements obtained with purified casein in 40% urea solutions to illustrate the experimental results. The urea concentration was 40 gm. in a 100 cc. of solution. The density of this 40% urea solution was 1.10. The measurements were made in a refrigerated chamber at 0.7° C. The results show that the osmotic pressures of the casein solutions are proportional to the concentration, within the limits measured. From the values obtained the molecular weight of casein was calculated, making use of the Morse and Frazier[4] equation and employing for the molal volume the mean molal volume of the urea solution. The average value obtained for the molecular weight of casein is 12,300. This figure agrees fairly well with the result calculated from the chemical analysis of casein.[5]

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[1] Hildebrand, J. H., Solubility, Chap. 14, Chemical Catalog Co., 1924.

[2] Sorensen, S. P. L., and co-workers, *Compt. Rend. Trav. Lab. Carlsberg*, 1915-17, xii, 262; Adair, G. S., *Proc. Roy. Soc.*, 1925, cviii, 627.

[3] Svedberg, T., and Nichols, J. G., *J. Am. Chem. Soc.*, 1926, xlviii, 3081.

[4] Morse, H. N., and Frazier, J. C. W., *Am. Chem. J.*, 1905, xxxiv, 1.

[5] Cohn, E. J., *Physiol. Reviews*, 1925, v, 359.

3810

Blood Lactates in Various Anoxemic States.

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In the course of an investigation on the general subject of the oxidative aspect of functional metabolism which is being carried on in this laboratory, it seemed desirable to test the validity of the hypothesis of A. V. Hill[1] that lactic acid serves as a "governor" of recovery oxidation. To this end two techniques have been developed for the production in the anesthetized dog of anoxemic states, which have been shown by Fletcher and Hopkins,[2] and others, to be the sole cause of excess tissue lactates. Throughout, dogs anesthetized by intraperitoneal injection of amytal were employed. Samples of blood for analysis were drawn from the femoral artery. The recently published method of Friedemann, Cotonio and Shaffer[3] was used for the determination of lactic acid in the blood so obtained.

Artificial Exercise. Two sheet brass electrodes were implanted in the subcutaneous tissues over the midcervical and sacral regions of the spinal cord. By means of faradic stimulation, a regular series of short, vigorous, tetanic contractions, involving the muscles of the trunk and extremities, were produced over a period of 15 minutes. During this intense muscular activity the oxygen consumption of the animal increased eight-fold. The observed changes in blood lactates are shown in Table I.

Subnormal Oxygen Supply. The animal was caused to rebreathe from a closed system, from which the CO_2 was absorbed and to which oxygen was added at a rate below that at which it was consumed by the anesthetized dog. Animals so maintained over periods up to an hour developed deep cyanosis and marked hyperpnea. The observed changes in blood lactates are shown in Table II.

Since the hyperpnea evoked in these experiments itself constituted fairly vigorous muscular activity, the possibility arose that the blood lactate increase might result from the contractile process rather than simple general anoxemia. Accordingly, the procedures were repeated on curarized dogs, artificial ventilation from the closed system being employed. The observed changes in blood lactates are shown in Table III.

We have shown that artificial exercise in the anesthetized dog produces rises in blood lactates comparable to those demonstrated by Hill and co-workers in man after voluntary muscular work. Furthermore, in one experiment, a definite increase of oxygen con-

TABLE I.

Blood lactates after artificial exercise.

(Figures give mg. of lactic acid per 100 cc. blood.)

Exp. No.	Control before exercise	Time after exercise				
		0 min.	15 min.	30 min.	45 min.	60 min
A 2	25.4	42.0	20.8	—	—	
A 3	23.9	51.6	58.5	51.0	—	
A 4	36.2	98.2	103.6	—	59.1	
A 5	19.5	42.5	47.7	—	29.9	
B 11	19.3	40.0	—	—	—	20.3

TABLE II.

Blood lactates after anoxemia.

Exp. No.	Control before anoxemia	Duration of anoxemia	Time after anoxemia in minutes					
			0	15	30	45	60	75
B 6	16.4	18 min	37.0					
		30 min.	29.4					
		48 min.	43.70					
		60 min.	51.30					
B 7	15.1	47 min.	61.81	—	42.47	—	—	26.5 after 2½ hrs.
B 11	19.3	51 min.	58.4	—	27.20	—	19.3	—

TABLE III.

Blood lactates after anoxemia in the curarized dog.

Experiment No.	Control before anoxemia	Duration of anoxemia	Time after anoxemia in minutes			
			0	15	30	45
B 9	14.3	23 min.	64.3			
B 10	29.3	21 min.	47.0			
		49 min.	54.6	79.2	—	47.5
	After curare and operation 33.2					

sumption over the resting value was noted after artificial exercise. These facts indicate a sufficiently close relation between the 2 forms of exercise to permit an analysis conducted by our technique to be of general application.

We have also shown that a subnormal oxygen supply results in a definite and considerable increase in blood lactates, and that this increase is not due solely to the functional metabolism of the respiratory muscles.

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[1] Hill, A. V., The Herter Lectures, 1924, p. 102.

[2] Fletcher, W. M., and Hopkins, F. G., *J. Physiol.*, 1906-7, xxxv, 247.

[3] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, lxxiii, 335.

3811

The Pharmacological Actions of Phenylethanolamine.

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Phenylethanolamine (*a*-phenyl-*b*-aminoethanol) is related chemically to tyramine, epinephrine and ephedrine According to the recent work of Alles[1] its circulatory stimulant efficiency would be higher than that of ephedrine. In view of this and because its general pharmacological action have not been previously studied it was thought desirable to make a survey of its possibilities. The product used was synthesized as the sulphate by Gr. Gordon A. Alles of the Clinic of Dr. George Piness, Los Angeles, who generously donated a large quantity to the laboratory. The method of synthesis eliminated contamination with its position isomer (*b*-phenyl-*b*-aminoethanol). The product consisted of white glistening crystals; was freely soluble in water, and had a melting point of 239-240°C.

The toxicity of phenylethanolamine is relatively low, the minimal fatal dose intravenously in white rats is about 0.14 gm. per kilo, which is the same as that of ephedrine reported by Chen.[2] This tendency to low toxicity agrees with the results of Alles[1] on guinea pigs and rabbits. Doses of 1 to 5 mg. per kilo intravenously in anesthetized dogs, cats and rabbits promptly raised the blood pressure from 25 to 103%, and accelerated the pulse to 42%, the effects persisting about 10 minutes, and hence qualitatively resembling tyramine. There seemed to be less cardiac depression with phenylethanolamine than with ephedrine. During the blood pressure rise the pupils dilated, while the hind leg and intestine usually were passively dilated, but occasionally were constricted. In cats, the pressor action was not reversed by ergot, but was abolished by cocaine during sensitization to epinephrine, resembling the actions of tyramine[3] and ephedrine.[4] In rabbits, cocaine diminished but did not abolish the rise of blood pressure. Circulatory effects from gastric administration were inconclusive. Respiration in cats and rabbits showed no constant changes, but in rats there was a marked increase in rate with sublethal doses.

Concentrations of 1:25,000 to 1:2,500 applied to strips of excised rabbit and cat intestine, using the Magnus method, caused an inhibition and fall of tonus, but either inhibition or stimulation with guinea pig intestine. The nonpregnant uterus of the rabbit and pregnant guinea pig uterus were stimulated by both the amine and epinephrine, while the nonpregnant cat uterus was stimulated by the amine but relaxed by epinephrine, thus again resembling tyramine and ephedrine. Bronchi constricted from histamine and pilocarpine were not effectively relaxed by phenylethanolamine or ephedrine, while epinephrine caused relaxation; additional tests are necessary. Administered subcutaneously and intravenously, phenylethanolamine was less effective than epinephrine, about as effective as tyramine, but more effective than ephedrine in preventing the experimental edema of paraphenylenediamine and equally as effective as epinephrine in dionine chemosis, but ineffective (also tyramine and ephedrine) against mustard oil chemosis and dermatitis. Local application of 1% concentrations in the nose of human subjects shrank congested nasal mucosae and turbinates promptly without objectionable irritation, the effects persisting 2 to 3 hours, thus resembling ephedrine.

Provisionally, the pharmacological actions of phenylethanolamine resemble those of tyramine and ephedrine more than epinephrine. Further tests are being made experimentally and clinically.

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[1] Alles, G. A., *J. Pharm. Exp. Therap.*, 1927, xxxii, 121.

[2] Chen, K. K., *J. Pharm. Exp. Therap.*, 1926, xxvii, 61.

[3] Tainter, M. L., and Chang, D. K., *J. Pharm. Exp. Therap.*, 1927, xxx, 193.

[4] De Eds, F., and Butt, E. M., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 800.

3812

Excretion, Distribution and Fate of Bismuth Under Clinical Conditions.

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The object of this study is to determine the limitations and usefulness, and, if possible, to establish better control, of bismuth therapy in syphilis by quantitative studies of the excretion of bismuth in urine and feces, its distribution in body fluids and organs, influence of various conditions on mobilization of stored bismuth, relationship of bismuth concentration and dosage to therapeutic efficiency, toxicity, etc., in human subjects, both convalescent and syphilitic. The plan includes also a study of various agents and conditions in experimental syphilis. Although the program of work is not yet completed, we desire to record certain results that have been obtained to date with 3 preparations, namely, metallic bismuth in suspension, potassium bismuth tartrate and bismuth salicylate used in single and multiple therapeutic doses by intramuscular injection.

Briefly summarized, the main results are as follows: With all preparations, urinary excretion, using Leonard's method[1] slightly modified and adapted to our material, is prompt, occurring on the second day after administration; the peak of excretion is reached at the end of about 12 days and then the excretion falls steadily but continues over long periods (weeks or months). The rapidity and quantity of bismuth excretion in urine appeared to be greatest with the tartrate (55% end of 53 days after 0.11 gm. Bi), probably less with the salicylate (uncompleted results) and least with the metal (24% end of 32 days after 1.2 gm. Bi.), while the order for duration is reversed. Increasing doses increase the excretion. The feces contain very little or only traces of bismuth, the incomplete excretion, therefore, being presumably due to storage in the body. This stored bismuth may be mobilized by certain conditions, for sudden marked increases in excretion have been demonstrated during attacks of gastric crises and of fever. External application of heat ("baking") to the whole body caused a moderate increase in urinary excretion. It would seem that the stored bismuth could be drawn upon for medication of the patient without the need of further administrations of bismuth. Other pyrogenic and various agents are being tried. Provisionally, bismuth acts as a diuretic without demonstrable injury to the kidney as indicated by the absence of albumin, casts, etc., but these features require further testing under controlled conditions.

Except for 12 cases of "bismuth-line" and 4 cases of mild stomatitis, no evidence of toxicity from metallic bismuth have been encountered among 100 individuals receiving a total of 1,500 injections, the highest number of injections in one individual being 131, equal to 26.2 gms. of bismuth in 18 months, the next highest being 119 injections (23.8 gm. Bi.) during the same period; nor after a total of 33 doses of the tartrate and 82 doses of the metal together (total 15 gm. Bi.) in 27 months and after 4 doses (0.3 gm.) of the salicylate. Hence, even with the supposed, considerable stores of bismuth in the body after these preparations, the danger of toxicity seems to be practically negligible.

Our results on distribution are too few to warrant a statement, but we have detected bismuth in cerebrospinal fluid and blood 10 months after administration of bismuth and expect to find it in all organs, but especially in the usual organs for storage of heavy metals. We are indebted to Dr. Frederick Proescher of the Agnew State Hospital for assistance in securing a portion of the materials used in the study. The work is being continued.

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[1] Leonard, *J. Pharm. Exp. Therap.*, 1926, xxviii, 81.

3813

Comparative Electrocardiography of Cardiac Drugs With Reference to Emesis from, and Distribution of, Digitalis.

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Electrocardiographic studies of pigeons were made with the idea of comparing the emetic and cardiac actions of digitalis, since emesis is a prominent action of the drug in pigeons, and the emetic assay method appears to predict the therapeutic dosage for slowing of the heart in man. Using tinctures of the drug injected intravenously, one-fourth the minimum emetic dose of digitalis caused a 10% slowing of the rate, and one-half the dose a 20% decrease in auricular rate and an A-V block in some pigeons. A single minimum emetic dose, causing emesis in 10 minutes, produced heart block or marked slowing in 1 to 5 minutes and 3 times the emetic dose produced all the effects in 10 to 15 seconds. The cardiac slowing was always preceded by a short period of acceleration which was due only partly to the injection fluid. On the other hand, veratrum and aconite, which also affect the heart through the vagus mechanism but do not cause emesis in pigeons, caused an immediate but brief slowing followed by tachycardia, either auricular or ventricular in origin, and in the case of veratrum there was, in addition, a curious waxing and waning of electrical complexes in cycles, 20 to 30 beats long. That the cardiac changes after digitalis and the slowing of veratrum were of vagal origin was shown by the fact that the effects were prevented and abolished by atropine and vagotomy. The effects were not altered by previous injection of ergotoxine, which by itself slowed the pulse through paralysis of the accelerator nerves, although epinephrine, in large doses, still caused a marked and prolonged acceleration presumably due to muscular stimulation. The electrocardiographic results with the cardiac drugs used corroborate their well known pharmacological actions and indicate practical possibilities of the method for studies of their distribution, accumulation and excretion in different species. An application was attempted in connection with a study of the distribution of digitalis with reference to emesis, with the following results.

Acetone extracts of organs from pigeons injected with digitalis were treated with normal saline solution, filtered and injected intravenously into healthy pigeons which were then observed electrocardiographically. Control injections of saline solution and of blank organ extracts gave only slight and temporary cardiac acceleration, while the addition of a single, minimal emetic dose of digitalis caused the typical effects of digitalis described above, but organ extracts after the injection of a single emetic dose were negative. Liver extracts from pigeons receiving from 1 to 6 minimum fatal doses of digitalis gave definite digitalis effects, while the heart and blood extracts were negative; lung extracts were variable. Hence, it appears that digitalis, even after very large and fatal doses, disappears rapidly from the circulation and is not detectable in the heart where it might be assumed to accumulate on the basis of current conceptions of the mechanism of digitalis emesis.[1]

The tendency to accumulation in the liver was supported by another series of experiments with organ extracts and in which emesis was the index of digitalis action. Here the amount of digitalis needed for positive effects from organ extracts after injection of the drug was 6 to 8 times the M. F. D., showing that the electrocardiogram was more sensitive than emesis as the index of digitalis action.

From these results and also from the facts that previous atropinization did not prevent emesis in pigeons and ergotoxinization and vagotomy together did not prevent nausea, it may be concluded that digitalis emesis is of peripheral origin.

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[1] Hatcher and Weiss, *J. Pharm. Exp Therap.*, 1927, xxxii, 37.

3814

Inactivation of Staphylococcus Bacteriophage by Trypsin.*

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It is already known that bacteriophages active on intestinal organisms exhibit a high degree of resistance to the action of trypsin. With the exception of a trypsin-susceptible Shiga bacteriophage reported by Wollman,[1] this resistance seemed to extend itself to bacteriophages in general. Indeed, Arnold and Weiss[2] were able by trypsinization to free the lytic filtrates of bacterial and other proteinaceous residue. During the course of similar studies in the laboratory of d'Herelle at Alexandria, the writer had occasion to trypsinize a variety of lytic filtrates with the object of freeing them of these protein residues. In the course of this work it was discovered that 2 "races" of staphylococcus bacteriophage were rendered inactive by trypsin within a period of about 48 hours. The 2 "races" in question had been isolated by Dr. Gratia of the Pasteur Institute at Brussels. One of them appeared to be active only on one strain of staphylococcus, whereas the other was capable of lysing a number of staphylococcus strains. Both were highly active and produced complete clarification of homologous bacterial suspensions, and this better at 33° C. than at 37° C.

The experiments were originally carried out as follows: A small quantity (2-5%) of powdered trypsin† was emulsified in a freshly lysed culture and the treated filtrate was then filtered through coarse filter paper to remove the larger particles from the suspension. To this treated filtrate was then added about 2% of chloroform to inhibit bacterial growth. A capillary tube of heated egg-white was dropped into the mixture to serve as an indicator of the progress of tryptic activity. These suspensions were incubated at 37° C., the optimum temperature for tryptic digestion, for periods of 48 hours or longer. As a rule the chloroform served to keep the suspensions free of bacterial growth, so that it was possible in most instances to test the lytic activity of the trypsinized filtrate at frequent intervals without preliminary filtration through a Chamberland candle. The safety of this direct procedure in the case of tubes which gave no macroscopic evidence of bacterial growth was elicited by transferring several drops of the chloroform treated suspensions to broth alone. At times, however, the trypsinized suspensions treated with chloroform showed well defined bacterial growth, and curiously, this was often most marked immediately above the surface of the chloroform in the bottom of the tube. Chloroform sometimes also produces a peculiar flocculation in Martin's broth, which must not be mistaken for bacterial contamination. A number of tests were also carried out without the use of chloroform, but on filtrates which immediately after trypsinization were passed through filter paper and then through a Chamberland candle.

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* Studies carried on as John Simon Guggenheim Memorial Fellow.

† Several brands of trypsin have been employed.

Under the conditions indicated, both "races" of staphylococcus bacteriophage appeared to be completely inactivated at the end of about 48 hours. Serial passages after this point failed to elicit any latent lytic activity. It is, however, important to carry the inactivation to its completion. When this is not the case the unlysed cultures are certain to reveal the lytic principle in its full potency in one or more serial passages. The nature and permanence of this inactivation by trypsin was not further determined. That it is not to be attributed to the chloroform is indicated by the fact that both bacteriophages were still active after 24 days exposure to chloroform alone. That it cannot be attributed to the action of trypsin on the staphylococcus used in determining the activity of the filtrate is indicated by the fact that these organisms grow abundantly in broth which has been recently trypsinized and filtered through a Chamberland candle. Moreover, the amount of trypsin, carried over into the bacterial suspensions from the trypsinized filtrate in conducting the test, is so small that it need not be considered. The possible influence of the trace of chloroform is ruled out by the analogous results with chloroform-free trypsinized filtrates.

A point of interest in connection with these studies is that freshly prepared staphylococcus suspensions in broth, which have received the trypsinized filtrate just before complete inactivation of the bacteriophage has been effected, may not show visible lysis in less than 48 hours or even 72 hours. Whereas control tubes having received the normal, untrypsinized lytic filtrate attain complete clarification within 24 hours; the tubes which had received the partially inactivated filtrate present at this time a heavy cloud of bacterial growth. The first impression one obtains is that the bacteriophage has at this point been definitely inactivated. That this need not necessarily be true is known to all students of bacteriophagy. A few serial passages may suffice to elicit a fully active bacteriophage from such unlysed cultures. The particular interest is that such heavily clouded unlysed cultures may begin to show complete lysis 48 to 72 hours later. In other words, these cultures do not begin to show clarification until long after the period of most active bacterial growth has passed. The peak of bacterial growth is probably attained well within the first 24 hours. These observations certainly do not harmonize with the concept that the d'Herelle phenomenon is necessarily closely linked with the period of logarithmic bacterial growth. That this delayed lysis is not due to a spontaneous autolysis of the staphylococcus is indicated by the fact that nothing of the sort has ever been observed in control tubes containing the organism alone, or the organism plus a comparable amount of trypsin. Moreover, it is of interest that the lysis begins at the top of the turbid suspension and gradually extends downward as a sharply defined column of complete clarification. This may be easily followed in cultures undergoing lysis at room temperature. These observations are in harmony with the recent observations of d'Herelle[3] on the accelerating influence of increased oxygen tension on the lytic activity of the bacteriophage.

A study of the influence of trypsin on various coli, typhoid and dysentery bacteriophages failed to reveal any susceptibility on the part of these bacteriophages. Daily retrypsinization of these filtrates over a period of more than 10 days has left them active and true to form. No natural bacteriophage was recovered from several batches of trypsin examined. A single experiment on plague bacteriophage in Dr. d'Herelle's laboratory showed this bacteriophage inactive after five days exposure to trypsin. Because the supply of trypsin at this time became exhausted and none was immediately available, this experiment could not be repeated. On his return home, about 5 months later, the writer found that the plague bacteriophage had spontaneously become inactive, suggesting that this bacteriophage is not nearly as resistant as those we are more familiar with. It is well known that bacteriophages may be preserved over a period of years, at any rate, without losing their activity completely.

The writer is not inclined to predict that all staphylococcus bacteriophages may likewise be inactivated by trypsin. The literature on the bacteriophage teaches us the unwisdom of making generalizations on the basis of isolated observations. It will, however, be of interest not only to extend these studies to other "races" of staphylococcus bacteriophages, but to bacteriophages active for other nonintestinal microbes. The single experiment on the plague bacteriophage suggests that this property may possibly extend to other bacteriophages.

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[1] Wollman, E., *Compt. rend, Soc. de biol.*, 1924, xc, 59.

[2] Arnold, L., and Weiss, E., *J. Infec. Dis.*, 1925, xxxvii, 411.

[3] d'Herelle, F., *Compt. rend. Soc. de biol.*, 1927, xcvi, 451.

New York Meeting.

Cornell University Medical College, January 18, 1928.

3815

The Composition of Bone. III. Physico-Chemical Mechanism.

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There have recently been two notable investigations of the physico-chemical mechanism of bone salt formation. Holt, La Mer and Chown[1] concluded that blood serum is normally supersaturated with $Ca_3(PO_4)_2$ to the extent of more than 200%. Sendroy and Hastings[2] also made careful determinations of $K'_{sp}Ca_3(PO_4)_2$ and of the solubility of this salt in serum, and obtained similar experimental results. They interpreted the results differently however, and concluded regarding supersaturation that "the evidence in every direction seems to be strongly opposed to this point of view," that "the data indicate that the theory of supersaturation is inadequate," that "the theory of complete dissociation of electrolytes . . . seems to fail as an adequate explanation for the solubility of calcium in serum." These investigations have thus demonstrated that the behavior of calcium and inorganic phosphorus in serum and in solutions is inexplicable when $K'_{sp}Ca_3(PO_4)_2$ is applied as the criterion for the precipitation of calcium phosphate.

When their data are studied from another viewpoint, more plausible explanations result. The empirical $Ca \times P$ product of Howland and Kramer[3] has proved so valuable that it seemed to us that it might have theoretical validity. This empirical product would have theoretical validity if the calcium phosphate were deposited as a result of a second order reaction such as $Ca^{++} + HPO_4'' \rightarrow \downarrow Ca\,HPO_4$.

If $Ca\,HPO_4$ is the substance first deposited then its solubility product is likely to be the quantity which determines whether or not precipitation shall occur. From data in the literature we have calculated that $pK_{sp}Ca\,HPO_4 = 6.4 \pm 0.1$ and $pK'_{sp}Ca\,HPO_4 = 6.4 - 2.3\sqrt{\mu}$ at 38° C.

The ion product $[Ca^{++}] \times [HPO_4'']$ has been calculated for inorganic serum solutions and serum on the basis of the complete dissociation of electrolytes. For such solutions containing 10 mg. Ca per 100 cc. and from 2.0 to 7.0 mg. inorganic P per 100 cc., the results range as follows: $[Ca^{++}] \times [HPO_4''] = 1.4 \times 10^{-6}$ to 4.8×10^{-6}, while $K'_{SP}CAHPO_4 = (3.2 \pm 0.8) \times 10^{-6}$.

Thus the ion products for $CaHPO_4$ *in such solutions are of the same order of magnitude as* $K'_{SP}CaHPO_4$. *Moreover, for solutions with empirical Ca × P products less than 35 the ion products are definitely less than* $K'_{SP}CaHPO_4$.

For the same solutions calculation gives $[Ca^{++}]^3 \times [PO_4]^2 = 0.7 \times 10^{-23}$ to 8.3×10^{-23}, while $K'_{SP}Ca_3(PO_4)_2 = 10^{-27}$ for solutions and $K'_{SP}Ca_3(PO_4)_2 = 2 \times 10^{-26}$ for serum.

Thus with a Ca × P product ranging from 20 to 70 the ion product for $Ca_3(PO_4)_2$ is about 1000 times greater than its K'_{SP} in serum and about 10,000 times as great as its K'_{SP} in inorganic solutions of the same ionic strength.

These otherwise inexplicable values may be accounted for if we consider that $K_{SP}Ca_3(PO_4)_2$ does not give the conditions necessary for the formation of $Ca_3(PO_4)_2$. This would be the case if $Ca_3(PO_4)_2$ could not be prepared by precipitation, but was formed only as a result of secondary reactions in the solid phase. A search of the literature of the calcium phosphates revealed not a single instance in which the precipitation of pure $Ca_3(PO_4)_2$ has been described. Bassett[4, 5] stated that it is a peculiar fact that this compound, discussed in all textbooks, has been known in neither the natural nor artificial condition, and that laboratory preparations vary considerably from the correct Ca/P ratio. The evidence is such as to arouse the suspicion that there may be no such chemical entity as $Ca_3(PO_4)_2$. But whether or not such a substance can be prepared in other ways, no one has succeeded in preparing it by direct precipitation. It has been repeatedly found that in acid solution the precipitate was always $Ca\,HPO_4$, and that in alkaline solution the precipitate always contained a larger proportion of lime than is required for the formula $Ca_3(PO_4)_2$. It has also been repeatedly demonstrated that the precipitated $Ca\,HPO_4$ reacts with the supernatant solution. This reaction is sometimes written $4\,CaHPO_4 \rightarrow Ca(H_2PO_4)_2 + Ca_3(PO_4)_2$ in which the primary phosphate formed goes into solution, leaving as the final solid phase $Ca_3(PO_4)_2$. This latter, however, is not a precipitate—the precipitate is the substance which is deposited from solution. The evidence which we have presented here indicates strongly that the initial solid phase is

Ca HPO_4 and that $K'_{sp}CaHPO_4$ is the quantity which governs the formation of the precipitate. If the solution is alkaline, Ca $(OH)_2$ may be deposited simultaneously with the Ca HPO_4.

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[1] Holt, L. E., Jr., La Mer, V. K., and Chown, H. B., *J. Biol. Chem.*, 1925, lxiv, 509.

[2] Sendroy, J., Jr., and Hastings, A. B., *J. Biol. Chem.*, 1927, lxxi, 783.

[3] Howland, J., and Kramer, B., *Trans. Am. Ped. Soc.*, 1922, xxxiv 204.

[4] Bassett, H., Jr., *Z. anorg. Chem.*, 1908, lix, 1.

[5] Bassett, H., Jr., *J. Chem. Soc.*, 1917, cxi, 620.

3816

The Composition of Bone. IV. Primary Calcification.

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Sixteen specimens of normal rat bone were analyzed using the micro-technique described in previous communications.[1, 2] The age of the rats ranged from 1 day to maturity. A mean value of 1.99 ± 0.05 was obtained for the ratio residual Ca/P for these normal bones. In the adult rats, carbonate calcium constituted 15 to 16% of the total calcium; in the very young rats this value was only 8 to 10%. Transition values of 12 and 13% were obtained in rats 6 and 9 weeks old respectively. Since the proportion of calcium carbonate to calcium phosphate varies with age, it is incorrect to assign a definite constant formula to bone such as $3\,Ca_3(PO_4)_2\,CaCO_3$. Bone apparently consists of at least two compounds whose proportions may vary independently. This is also borne out by the results reported by Howland, Marriott and Kramer.[3] They found that the bones of rachitic rats contain a smaller proportion of calcium phosphate to $CaCO_3$ than do the bones of normal rats.

Twenty-five rachitic rats were fed cod-liver oil concentrate for 8 days. The rats were then autopsied and the "lines" of freshly deposited lime salts were removed and analyzed. The adjacent epiphyses and diaphyses were also removed and analyzed. Although the ratio for normal rats with this method had been established as described above, the shafts of the leg bones of the same animal were analyzed as an additional control. The values for residual Ca/P obtained were: 2.05 for the shafts; 2.10 for the epiphyses and diaphyses; and 2.38 for the test lines.

A similar study using irradiated yeast instead of cod-liver oil concentrate gave similar results. In this case care was taken to remove only fresh calcification from the metaphyses. The values obtained were: 2.05 for the shafts; 2.25 for the metaphyses; and 2.16 for the test lines. Test lines in which the healing was induced by feeding irradiated cholesterol to rachitic rats gave a ratio of 2.24.

Another series of rats was fed 1% butter in addition to the rickets producing diet for 4 weeks. Fresh calcification was obtained in the metaphysis, but not in the provisional zone. The ratios obtained were 1.94 for the shafts and 2.14 for the metaphyses As a further control the shafts of a normal rat of the same age as the test rats were analyzed and gave a value of 2.03.

The carbonate calcium constituted 15 and 16% of the total calcium in the shafts of the test rats. In these same rats, however, the fresh calcification contained only from 10 to 12% carbonate calcium. Thus the proportion of carbonate calcium in the older bones is 30 to 50% higher than in the fresh deposit. This is similar to the difference between the composition of the bones of older rats as compared with very young rats.

The values for the ratio resid. Ca/P in the specimens of fresh calcification were calculated from mean values of Ca and P. These mean values were obtained by averaging the results of repeated analyses. In one case, for example, 10 determinations of calcium and 12 determinations of phosphorus were made. The high values of the ratio obtained, therefore, do not appear to be ascribable to experimental error, since a sufficient number of determinations were made to rule out accidental high values of calcium and low values of phosphorus.

The results are interpreted as showing that primary calcification has a different empirical composition from older bone in that it contains a larger proportion of calcium than the latter. In the light of the mechanism discussed in the preceding communication[4] it seems probable that this excess calcium is present as calcium hydroxide and that the phosphate is present as $CaHPO_4$.

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[1] Shear, M. J., and Kramer, B., *J. Biol. Chem.*, 1927, lxxiv, ix.

[2] Kramer, B., and Shear, M. J., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 141.

[3] Howland, J., Marriott, W. McK., and Kramer, B., *J. Biol. Chem.*, 1926, lxviii, 721.

[4] Shear, M. J., and Kramer, B., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 283.

3817

Histopathology of Experimental Vesicular Stomatitis of the Guinea Pig.

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In a previous article by one of the writers,[1] the histopathology of the lesions of vesicular stomatitis was described and mention was made of further studies on this subject, which were in progress at the time. Additional facts have been secured from the later investigations and are herewith presented.

The tissues examined consisted of pads from guinea pigs, 18 to 120 hours after inoculation with guinea-pig passage virus of vesicular stomatitis of horses. The pads were fixed in Zenker's fluid (with acetic acid) and stained with eosin-methylene blue.

The cells which are most profoundly affected are those in the upper part of the epidermis, that is, just beneath the horny layer. The basal epithelial cells are not as much damaged by this virus as by the virus of herpes. The cells immediately beneath the horny layer are swollen and stain less intensely than normal ones. As degeneration proceeds vesicles form, containing a loose reticulum composed of collapsed degenerated cells. Later there is infiltration with polymorphonuclear and endothelial leucocytes.

The cytoplasm of the affected epithelial cells becomes granular. The nuclei swell and finally appear as empty sacs with a dark blue-staining membrane. The nucleoli become first vacuolated and later fragmented. Often they are extruded and enter the cytoplasm, and frequently the staining is pink instead of blue.

Intranuclear inclusion bodies are also present. It should be mentioned that the criteria employed for the recognition of these structures vary with the investigator. We have accepted the criteria of Lipschütz.[2] Two types of inclusions are distinguishable: one consists of small eosin-staining bodies which are frequent and conform to those described by Gins.[3] They are found not only in the specific lesions, but also in lesions induced by agents other than the virus (Trautwein,[4] Ruhle,[5] and the writers). The second consists of medium-sized eosin-staining bodies, 1 to 2 microns in diameter, which are usually present in greatest numbers within 48 hours after the inoculation. They are regular in outline and are highly refractive. Some of them resemble the nuclear bodies of varicella and of herpes. Minute study, however, indicates that they differ from the latter, so that the question has arisen whether or not they represent degenerated nucleoli which stain pink instead of blue. It should be added that indistinguishable lesions of the epithelial cells and nuclear inclusions have been found in experimental foot-and-mouth disease of the guinea pig.[6]

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[1] Olitsky, P. K., *J. Exp. Med.*, 1927, xlv, 969.

[2] Lipschütz, B., *Arch. Dermat. u. Syph.*, 1921, cxxxvi, 428.

[3] Gins, H. A., *Centr. Bakt.*, 1 Abt., 1922, lxxxviii, 265.

[4] Trautwein, K., *Arch. f. wiss. u. prakt. Tierheilk.*, 1925, lii, 475.

[5] Ruhle, F., *ibid.*, 1926, liv, 197.

[6] For some of the specimens of foot-and-mouth disease tissues, we are deeply indebted to Dr. F. C. Minett.

3818

The Transfer of Normal and Irritated Omentums with Subsequent Streptococcus Immunity in Rabbits.

R. W. LINTON. (Introduced by F. P. Gay.)

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In a recent paper[1] it was shown that if areas of granulation tissue are formed in the peritoneums of rabbits, these animals become immune to a subsequent intrapleural infection with a highly virulent hemolytic streptococcus. The intrapleural doses varied from 100 to 250 M.L.D. and successful recovery occurred in 75% of the cases. The protection appeared to be due to the migration of tissue macrophages or clasmatocytes from the peritoneal granulating areas into the pleural cavity and particularly into the subserous tissues of the diaphragmatic and parietal pleura.

The present report describes an extension of the transfer technique. A portion of the peritoneal granulating area is removed and inserted in the peritoneum of a normal rabbit. The method is as follows: A peritoneal focus of cells is built up in a rabbit by two or three intraperitoneal injections of an irritating substance, in this case a mixture of aleuronat and starch. Four days after the last injection the animal is sacrificed, and its omentum removed and carefully freed from any lumps of aleuronat which may have become adherent to it. It is then immediately inserted in the peritoneum of a normal rabbit, under complete anesthesia. An omentum of this type is at least twice normal size. Microscopically, it is found to contain many times the number cells in the normal omentum. This increase is due principally to cells of the tissue macrophage or clasmatocyte type, as may be shown in animals stained with Trypan Blue.

At various intervals from 2 to 7 days after the insertion of the omentum, these animals were infected in the right pleural cavities with *Streptococcus hemolyticus,* varying in amount from 20 to 100 M.L.D. Sixty per cent of these animals were found to be protected. These experiments were controlled by the intrapleural infection of rabbits into whose peritoneums normal omentums had been inserted, and in some cases by the simultaneous infection of normal rabbits. No protection was afforded by the normal omentums, and the normal animals also died with typical infections. Histologically, rabbits which have recovered from intrapleural infections after omental transfer show in the serosal linings of their pleural cavities an increased number of tissue macrophages. Furthermore the percentage of clasmatocytes in the pleural exudates is somewhat higher than normal: 96% in contrast to the normal count of 85%.

A preliminary experiment has been conducted, using guinea pigs' omentums as the transferred material. Irritated omentums, prepared as in the case of the rabbits, from 2 pigs were transferred to each rabbit. Four days later these animals were given 20 M.L.D. of *Streptococcus hemolyticus* intrapleurally. Two of these animals survived and 2 died. The control rabbit, which had 2 normal guinea pig omentums, died with a typical empyema. The protection afforded by this method appears to exist only when relatively small doses of the infecting organism are given, *i. e.,* up to 50 M.L.D. When 50 to 100 M.L.D. are used the protection is inconstant, and beyond 100 M.L.D. is non-existent. Omentums which have been stained with Trypan Blue, during the course of the irritation, protect as well as unstained tissues.

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[1] Linton, R. W., *Arch. of Path. and Lab. Med.,* in press.

3819

Heteroplastic Transplantation of Halves of Girdle Rudiment in Amblystoma.

JOSEPH L. SCHWIND. (Introduced by J. S. Nicholas.)

From the Osborn Zoological Laboratory, Yale University.

In heteroplastic transplantations of the eye, Harrison[1] found that when the lens came from the donor species and the retina from the host species, each part exerted an influence on the other's growth. In order to see if a mosaic system would react in a similar way when the rudiment contained tissue from the two species, halves of the girdle rudiment were transplanted between *Amblystoma punctatum* and *A. tigrinum*. Reciprocal transplants of dorsal, ventral, anterior and posterior halves of the girdle rudiment were made. Except in a few cases killed for the study of intermediate stages, all animals were raised until the *tigrinum* of the pair had a fully formed fourth digit, usually 70 to 80 days after operation.

The limbs and girdles, both normal and operated, were prepared by a modified van Wihje method for the staining of cartilage, and after the muscles had been carefully removed, were mounted in damar. It was found that at 80 days there are well marked differences between the normal girdles of the two species. The *punctatum* girdle is smaller, has undergone considerably more chrondrification than that of the *tigrinum*, has a rounded coracoid and a short, stout scapula which passes gradually into the broader suprascapula. The *tigrinum* girdle is larger, having its coracoid shaped more like a plow, and having a very long, slender scapula at the top of which is a much broader suprascapula.

The specificity of the transplanted tissue was shown by the fact that there was no regulation of form due to transplantation into the girdle region of the host species. The portion of the operated girdle that had come from the donor could easily be recognized. The procoracoid and the muscles attached to the operated girdles were so variable that they could not be studied.

In the dorsal half transplantations, which included the rudiments of the free limb, scapula and suprascapula, these parts were always of the donor species. Under optimum conditions, the transplanted limbs grew to the same size upon the host species as their control limbs did upon the donor species. The growth in size of the limbs is due to factors intrinsic to the graft. Cell counts showed hypoplasia of the spinal ganglia supplying the grafted limb in the case of a *tigrinum* bearing a *punctatum* limb. In the reverse combination, a *punctatum* bearing a *tigrinum* limb, there was hyperplasia of the ganglion cells. The effect upon the ganglia of *A. punctatum* was relatively greater than upon those of *A. tigrinum,* as was expected from the size of the limbs.

In the ventral half transplantations, which include the rudiments of the coracoid and procoracoid, the free limb, scapula and suprascapula are always of the host species. This is the only series in which complicating factors are not introduced by the presence of either a larger or smaller limb than normally would be present. It shows that the transplanted tissue from the donor species has no effect upon that part of the girdle supplied by the host species, for the scapula and suprascapula in all cases are of a size normal to the host. This series indicates that the tissue which has the potentialities of forming girdle material is more extensive than has hitherto been supposed.

In the transplantations of the anterior and posterior halves, the dividing line being taken as the projection of the fourth-fifth somite boundary, many of the limbs are compound; tissue from both species is present in the limb at the same time and capable of morphological identification. This is especially striking in the digits and carpus. In single limbs which contain tissue from both species, if the grafted tissue formed the anterior half, the anterior digits are of the donor species and the posterior digits are of the host species. If the posterior half of the rudiment was transplanted, the posterior digits are of the donor species and the anterior ones are of the host species. This shows that the tissue which is determined to form the digits is localized in the limb disc in an antero-posterior direction, the material for the first digit lying most anteriorly. With a few exceptions, the carpal elements are similarly affected. That is, a hand having the first two digits composed of *tigrinum* tissue will have carpales 1 and 2 and the radiale enlarged, while a hand having the first two digits composed of *punctatum* tissue will have carpales 1 and 2 and the radiale reduced.

The girdles of the posterior half series were very little or not at all affected by the operation, making it probable that there is little or no girdle-forming material posterior to the fourth-fifth somite boundary.

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[1] **Harrison, Ross G., *Anat. Rec.*, 1925, xxxi, 299.**

3820

Agglutinins and Protective Bodies in Pneumococcus Immunity and Application to the Control of Specific Therapy.

W. D. SUTLIFF. (Introduced by R. L. Cecil.)

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Noble[1] has described an accurate method of determining agglutinins in the blood within 2 minutes after setting up the test. Since agglutinin response in pneumonia has been found to be fairly well correlated with the clinical course of the disease, in untreated cases by Blake[2] and in serum treated cases by Cole,[3] we felt that a thorough trial of this new method of agglutinin determination was indicated.

In several patients with lobar pneumonia we have correlated the appearance of agglutinins with the dosage of serum and with the appearance of protective bodies in the patient's blood serum and we present these results pending the examination of a larger series. In 1 case of lobar pneumonia caused by pneumococcus Type IV, small doses of Felton's concentrated anti-pneumococcus serum (polyvalent against Types I, II and III) were given at varying intervals, and samples of blood serum taken at corresponding intervals were examined for agglutinins and protective bodies against the 3 fixed types of pneumococcus.

TABLE I.

Agglutinin titer and mouse protective substance.

	Pneumococcus—Type I.		Type II.		Type III.	
	Agglutinin titer	Mouse protect'n (MLD)	Agglutinin titer	Mouse protect'n (MLD)	Agglutinin titer	Mouse protect'n (MLD)
Before serum	00	00	00	00	00	00
30 min. after 1 cc.	00	10	00	1,000	00	10
30 min. after 5 cc.	00	100,000	00	100,000	00	1,000
30 min. after 5 cc.	1:20	100,000	00	100,000	00	1,000
30 min. after 5 cc.	1:20	100,000	00	1,000,000	1:20	1,000
30 min. after 5 cc.	1:100	10,000	00	1,000,000	1:40	(Tests incomplete)
90 min. after 5 cc.	1:40	10,000	1:10	1,000,000	1:40	
3 hours after 15 cc.	1:80	10,000	1:20	1,000,000	1:100	1,000

From these figures we may conclude that following the administration of Felton's polyvalent serum, agglutinins appear after a certain amount of protection has been established.

A further comparison has been made of agglutinins and protective bodies in specimens obtained at a time when a fall in antibodies was taking place. A fall in agglutinin titer took place during the convalescence of a patient with Type I lobar pneumonia during the acute phase of which he received a considerable amount of Type I serum. No decrease in protective bodies occurred during the same interval. In another Type I case treated with Felton's serum, agglutinins, once present, disappeared entirely, but protective antibodies for pneumococcus Type I remained about the same in amount in the same samples of serum. In still another case of pneumococcus Type IV pneumonia treated with polyvalent serum, the disappearance of agglutinins, but peristence of protective antibodies against pneumococcus Type I was demonstrated.

To illustrate the application of this test in guiding specific therapy a case of Type II pneumonia may be cited in which a specimen of patient's blood before the administration of therapeutic serum showed no agglutination or protection. Specimens after the injection of 50 cc. of Felton's serum showed agglutination in 1:20 dilution. Protection tests done later showed the presence of considerable protection in the same blood serum sample. The maximum serum dosage was no longer necessary, thus saving annoyance to patient and saving serum. The case mentioned represents the majority of instances that are met, but more urgent circumstances may be shown by the following example. A moribund patient with a precipitin test in the urine for pneumococcus Type I showed no agglutinins in the first serum sample. Twenty cubic centimeters of serum were given. Two hours later a blood sample was taken and 30 cc. more of serum injected. This blood sample showed agglutinins in a dilution of 1:40. Another blood serum taken one-half hour after the second injection of therapeutic serum showed an agglutinin titer of 1:100. Mouse protection tests on the same samples of the patient's serum showed no protection before the administration of Felton's serum, but the presence and increase of protective bodies for pneumococcus Type I after the administration of Felton's serum. This reassurance was obtained quickly enough with this rapid agglutinin test to guide the further administration of serum.

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[1] Noble, Arlyle, *J. of Bact.*, 1927, xiv, 287.

[2] Blake, F. G., *Arch. Int. Med.*, 1918, xxi, 779.

[3] Cole, Rufus, *J. Exp. Med.*, 1917, xxvi, 453.

3821

The Mechanism of the Glycosuria of Acute Uranium Nephritis.

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A glycosuria occurring early in the course of severe acute uranium nephritis is a matter of common observation, but its mechanism is by no means clearly understood. The appearance of the urinary sugar has almost universally been attributed to the high blood sugar levels that are frequently observed in this type of nephritis, although Frank[1] believed that glycosuria might occur while the blood sugar was normal.

In our series of experiments 11 fasting female dogs were each given a single subcutaneous injection of 15 mg. uranium nitrate per kilogram of body weight. Four of the animals were killed with chloroform when the glycosuria was estimated to be at its greatest height, while the remaining 7 were permitted to live until they died with uremic manifestations. The blood sugar, blood non-protein nitrogen, total blood solids, urinary sugar and nitrogen, water ingestion, and urinary secretion were accurately determined at frequent intervals both before the administration of the uranium and throughout the subsequent life of the animals.

It was found that the glycosuria accompanied by a polyuria made its appearance 6 to 10 hours after the injection of uranium, reached its greatest height in about 28 hours, and lasted 9 to 44 hours. Throughout the course of the glycosuria the blood sugar remained normal or fell to a level slightly below that of the pre-uranium period. None of the blood sugar values observed during the glycosuria were suggestive of a hyperglycemia.

However, during the period of anuria, partial or complete, which closely followed the cessation of the glycosuria and persisted until the death of the animals, blood sugar values distinctly above the normal were found, but in no instance was the hyperglycemia accompanied by any glycosuria.

It seems probable that the glycosuria, which unquestionably is of renal origin, is due to the involvement of the kidney tubules, whereby the glucose normally present in the glomerular urine is prevented from being completely reabsorbed. Histological sections of the kidneys from the four animals killed during the height of glycosuria showed extensive tubular damage but no demonstrable pathological changes in the glomeruli while the kidneys of those animals which died in uremia showed extreme degeneration of both the glomeruli and the tubules. Calcification of the tubules which has been considered pathognomonic of bichloride poisoning occurred in the kidneys of one animal which had been totally anuric for six days prior to death.

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[1] Frank, E., *Arch. exp. Path. u. Pharm.*, 1913, lxxii, 387.

3822

Availability of Carbon Compounds for the Streptococcus.

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From the Laboratory of Biological Chemistry of Columbia University, College of Physicians and Surgeons.

The synthetic media described in our earlier papers[1, 2] have been used for testing the value of various substances as nutrients. The studies in this paper are concerned with the effect on the growth and viability of streptococci, of the addition of various carbon compounds to the media. These compounds include dextrose, galactose, mannite, lactose, maltose, sucrose, raffinose, glycerol, lactic, tartaric, malic and citric acids.

The experiments show: (1) In most cases dextrose shortens the period of viability markedly. Apparent increases in viability due to the addition of dextrose have been obtained but they were too small to be taken into account. (2) A comparison of dextrose with other sugars in reference to their availability for growth gives series such as sucrose > lactose, maltose > raffinose > mannite, dextrose.

There are several references in the literature to the growth value of dextrose. Samkow[3] and Waksman and Joffe[4] found that although dextrose did no harm, it did not aid materially. Frankel and Pyle[5] showed that dextrose retarded growth slightly. Koser and Rettger[6] reported glycerol better for growth than dextrose because less acid is formed and the organism is therefore "freer to develop". None of these investigations refer specifically to the streptococci. The only definite allusion to these organisms with respect to their utilization of dextrose was found in the communication made by Gordon.[7] He states that dextrose is not utilized by streptococci. Our results corroborate this.

(3) Of the several sugars tested, sucrose seems to be the most easily utilized, though in some cases it was without effect. (4) Similar results were obtained from glycerol, lactic, malic, tartaric and citric acids. These compounds are more often beneficial than the carbohydrates.

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[1] Krasnow, F., and Rosenberg, M. L., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 215-17.

[2] Krasnow, F., and Rosenberg, M. L., *J. Bact.*, 1926, xii, 385-408.

[3] Samkow, S., *Centralblatt für Bact., Parasit. und. Infect.*, 1904, xi, 305.

[4] Waksman, S. A., and Joffe, J. S., *J. Bact.*, 1922, lxxvii, 239.

[5] Frankel, F. H., and Pyle, E., *J. Infec. Dis.*, 1919, xxiv, 17.

[6] Koser, S. A., and Rettger, L. F., *J. Infec. Dis.*, 1919, xxiv, 301.

[7] Gordon, M. H., *J. Royal Army Med. Corps*, 1917, xxviii, 371.

3823

The Mechanism of the Lipemia of Bleeding.

ELLA H. FISHBERG AND ARTHUR M. FISHBERG.
(Introduced by I. N. Kugelmass.)

From the Chemical Laboratory of Beth Israel Hospital, New York City.

It has been known since the work of Boggs and Morris[1] that repeated and copious bleeding of rabbits produces a high grade lipemia. Similar lipemia may occur in humans following severe hemorrhages (Feigl[2]). Boggs and Morris[1] believe it possible that "the great loss of tissue proteins might have some influence on the abnormal fat metabolism" and that "lowered oxidation following a great loss of red cells plays a part." Sakai[3] and Horiuchi[4] think that the decrease in blood lipase may be the cause of the lipemia. Bloor[5] believes that nothing definite can be said as to the cause of the fat mobilization.

To further elucidate the mechanism of lipemia of bleeding, having in mind certain analogies with the lipemia observed in human nephrosis, we produced lipemia in 9 rabbits by bleeding, and compared the changes in total fats and cholesterol of blood with the alterations in the blood proteins, certain inorganic constituents and red blood cells. The animals were kept on the usual laboratory diet of cabbage, oats, and bread. The blood was obtained by incision of the rabbit's ear. About 35 cc. daily were removed, omitting a day occasionally when the animal seemed very weak.

The results in all 9 animals were essentially the same, and are illustrated in Table I.

TABLE 1.
Rabbit No. 4. Daily Blood Samples.

Blood drawn cc.	Sugar mg.%	NPN mg.%	Total Prot. gm.%	Alb. gm.%	Glob. gm.%	A/G	Chol. mg.%	Total Lipins mg.%	R. B. C. millions
33	150	30	6.37	4.09	2.28	1.8	161	479	4.5
35	140	30	6.50	4.06	2.44	1.7	152	552	3.5
25	115	33	6.15	4.10	2.05	2.0	181	482	2.5
30	120	29	6.28	4.16	2.12	1.98	149	621	1.8
29	122	31	6.55	4.04	2.51	1.6	165	581	2.0
20	124	30	5.905	3.881	2.03	1.91	157	672	2.5
26	124	30	5.55	3.71	1.84	2.01	191	821	1.9
30	115	30	5.43	3.59	1.84	1.95	184	721	2.0
26	112	27	3.94	2.61	1.33	1.99	254	1250	1.2
25	115	30	3.33	2.01	1.32	1.52	297	2157	1.2
30	140	30	4.03	2.21	1.82	1.21	382	2870	1.8
15	125	29	3.72	1.96	1.76	1.11	309	3120	1.5
26	123	30	3.80	1.84	1.96	0.94		3860	1.75
35	122	27	3.23	1.54	1.69	0.91	320	4120	1.4
35	110	29	3.40	1.71	1.69	1.01	354	3890	1.34
35	105	30	4.03	2.12	1.91	1.11	402	3540	1.1
15	115	42	3.33	1.69	1.64	1.0	408	3710	0.9
20	129	44	3.97	2.12	1.85	1.13	403	3140	0.65

Exitus 8 P. M. 6/21/27.

It is seen that as the protein content of the plasma diminished, the total lipins and cholesterol increased. During the first few days the drop in protein was slight, as was the rise in cholesterol and total fats. But when the proteins dropped notably there occurred a tremendous rise in total lipins and a moderate one in cholesterol. It is further seen that the red blood cells diminished in number from the very start. But the correlation of the rise in fats with the fall in red blood cells is not nearly so close as with the fall in proteins, for during the first few days the red blood cells decreased abruptly while the cholesterol and total lipins showed comparatively little change. In one rabbit (rabbit 8) which had an initially low blood protein content, though a normal red cell count, severe lipemia was present after only 4 days of bleeding.

Table II shows the initial and lowest protein concentrations and the concentrations of total lipins and cholesterol on the same days.

It is evident that the rise in total lipins is much greater both absolutely and in proportion to the initial value than that in cholesterol. This is also true of human lipemia as encountered in diabetes and nephrosis.

TABLE II.

Rab.		Initial					On day of lowest protein					
No.	T.P.	Alb.	Glob.	A/G	T.L.	Chol.	T.P.	Alb.	Glob.	A/G	T.L.	Chol.
4	6.37	4.09	2.28	1.8	479	161	3.23	1.54	1.69	0.91	4120	320
5	5.91	3.96	1.95	2.03	530	111	3.56	2.28	1.28	1.18	3450	380
7	5.30	3.67	1.63	2.25	390	109	3.10	2.09	1.01	2.1	3200	309
8	4.1	2.75	1.35	2.1	870	139	2.32	1.27	1.05	1.2	2400	301
9	5.75	3.75	2.0	1.85	580	106	2.2	1.15	1.05	1.1	4120	310

Table II also shows that the drop in albumin is greater than that in globulin, resulting in a decrease in the albumin/globulin ratio, once even in inversion. This is presumably due to the rapid regeneration of globulin as previously demonstrated by Kerr, Horowitz and Whipple.[6] The drop in colloid osmotic pressure of the plasma is thus greater than would be anticipated from measurements of the total proteins only, for a given concentration of albumin exerts a greater colloid osmotic pressure than an equal concentration of globulin. In fact, it has been shown by direct measurement that the colloid osmotic pressure per gram per cent of plasma protein is proportional to the albumin-globulin ratio.

The rise in plasma fats and lipoids more closely correlated with the fall in plasma proteins than with the diminution in the number of red cells. It seems highly probable that the lessened protein content of the plasma is intimately concerned in the lipemia of bleeding. It is possible that the accompanying lipemia is to be viewed as a compensatory mechanism, the organism mobilizing fatty acids, cholesterol and lecithin in an effort to make up for the diminution in colloid osmotic pressure that results from the loss of plasma protein. If this view is correct, lipemia is a manifestation of the organism to maintain the colloid osmotic pressure of the plasma, as various other devices are used to keep other physical and chemical properties of the blood near a constant level.

The fats are mobilized not only from the food but also from the fat depots. Thus, though some of our animals lost comparatively little weight during the experiments (from 2040 gm. to 1870 gm. after 15 days of bleeding), the fat depots of the body (subcutaneous, retroperitoneal, etc.) were almost completely devoid of fat. Nor was there any notable fatty deposition in the liver, kidneys, adrenals or other viscera. The animals become lipemic even if they receive almost no fat in the diet, though more slowly than when they are given fats.

No edema was noted at any time despite the drop in plasma proteins. However, the fact that some of the animals lost little weight despite the disappearance of the fat tissues quite possibly indicates that water was retained, though not sufficient to produce demonstrable edema.

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[1] Boggs and Morris, *J. Exp. Med.*, 1909, xi, 553.

[2] Feigl, *Biochem. Z.*, 1921, cxv, 63.

[3] Sakai, *Biochem. Z.*, 1914, lxii, 387.

[4] Horiuchi, *J. Biol. Chem.*, 1920, xliv, 363.

[5] Bloor, *J. Biol. Chem.*, 1921, xlix, 201.

[6] Kerr, Horowitz and Whipple, *Am. J. Physiol.*, 1918-19, xlvii, 356.

3824

Effect of Radium on Pharmacological Properties of Mercurochrome.

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The effect of various radiations from radium on Mercurochrome was studied by irradiating both the solutions and the solid crystals of the drug. The action of alpha and beta radiations was studied by crushing glass globules containing radium emanation in doses of 4 to 10 millicuries directly under solution of 2% Mercurochrome in water and immediately stoppering tightly the 10 cc. vials of the solution. The effect of gamma rays was studied by placing vials of Mercurochrome solution in juxtaposition to large quantities of radium. The irradiations of solid Mercurochrome crystals was performed in the same way. The pharmacological and bacteriological properties of the solid crystals after irradiation were studied by making a 2% solution of the same. Bacteriological studies were made in the following manner: Cultures of *B. typhosus* were used; the method of studying germicidal action was the so-called Hygenic Laboratory method. The temperature of medication was 20°; the proportion of culture and disinfectant used for making dilutions were 0.1 cc. in 5 cc. Subculture medium was also that of the Hygenic Laboratory method. Quantity used in each tube was 10 cc. Temperature of the incubator was 37°. Various dilutions of the drugs were employed. Time of exposure of the culture to the action of the disinfectant was in one series of experiments 5 minutes and in a second, 15 minutes. Control experiments with unradiated Mercurochrome solutions from exactly the same stock were made in exactly the same way. It was found that when solutions treated with the alpha and beta radiations were examined within 24 to 48 hours, there was a slightly increased bactericidal activity shown after 5 minutes exposure to the disinfectant. This increased bactericidal property of the solution, however, disappears after a few days. The results obtained must be ascribed to the presence of actual alpha particles in the solution, which particles gradually disappear on standing. Very intensive irradiations were performed with gamma rays by exposing both solutions and crystals of Mercurochrome to 3500 millicuries from 1 to 10 hours. An examination of all these samples bacteriologically on cultures of *B. typhosus,* as described above, revealed no increase in the potency of the Mercurochrome, nor a decrease in the same.

Pharmacological studies of the irradiated samples were made on living seedlings of *Lupinus albus.* In this case, as in the bacteria, it was found that exposure to alpha particles produced a slightly increased toxicity of Mercurochrome. This, however, was not due to any change in the drug, but to the presence of the particles themselves, because the increased toxicity disappeared after a few days. Pharmacological tests on animals showed also no change in the potency of Mercurochrome after the various irradiations. The results of all the experiments lead to the conclusion that radium radiations do not affect the potency of Mercurochrome at all, and indicate that the compound is certainly very stable as far as these radiations are concerned. The effect of Ultra Violet rays has already been described by Macht and Hill elsewhere.[1] The authors are indebted to Dr. Fred West of the Howard A. Kelly Hospital for the irradiations experiments.

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[1] Macht, D. I., and Hill, J. H., *J. Am. Pharm. Assn.*, 1927, xvi, 2.

3825

Adrenal Gland in Wild Gray and Albino Rat: Cortico-Medullary Relations.

JOHN C. DONALDSON.

From the Department of Anatomy, School of Medicine, University of Pittsburgh.

The wild gray rat *(Mus norvegicus)* has larger adrenal glands for a given body weight or body length than does the tame albino rat *(Mus norvegicus* var. *albinus)*.[1] In both varieties the females have larger adrenals than the males of equal size. In both sexes the adrenal glands of the adult gray rats weigh roughly twice as much as those of the albino rats. The work here reported was done to determine whether or not the proportion of the adrenal gland formed by the medullary (chromaffin) tissue was the same in both varieties or whether the larger glands of the gray rat consisted more largely of cortical cells.

The data for the albino rats were based on 27 female and 3 male adults. The material for the wild gray rats consisted of 9 females and 4 males caught near the laboratory and killed within 48 hours of capture. The glands were removed immediately after death, fixed, sectioned, stained, and the proportion of the glands formed by each part determined by measuring sections at every 100 micra.[2, 3] The results are shown in Table I.

TABLE I.

Percentage of the entire gland formed by the medulla.

Sex	Average Bd. W.	Wild gray medulla	Average Bd. W.	Albino medulla
Females	265 gm.	4.4%	167 gm.	6.2%
Males	229 gm.	5.9%	203 gm.	8.3%

Table II is the comparison between 4 hypothetical animals of the same body weight to show the number of mg. of cortical and medullary tissue that would be found, according to Table I, in their adrenal glands. The data for the weight of the adrenal gland compared to body weight are taken from "The Rat",[4] tables 148 and 206.

TABLE II.

Animal	Bd. Wt. gm.	Total wt. of adrenal mg.	Wt. of cortex mg.	Wt. of medulla mg.	% formed by medulla
Albino ♀	188	50.0	47.1	3.1	6.2
Albino ♂	188	33.1	30.3	2.8	8.3
Gray ♀	188	97.0	92.7	4.3	4.4
Gray ♂	188	80.0	75.3	4.7	5.9

This comparison indicates that in the adrenal glands of gray rats and albino rats of the same body weight (1) the proportion of medullary tissue to cortical tissue is less in the wild gray than the albino, (2) the absolute amount of chromaffin tissue is greater in the wild gray, and (3) most of the greater mass of the adrenals in the wild gray consists of cortical material.

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[1] Hatai, S., *Anat. Rec.*, 1914, viii, 511.

[2] Jackson, C. M., *Am. J. Anat.*, 1919, xxv, 221-289.

[3] Donaldson, J. C., *Am. J. Anat.*, 1919, xxv, 291.

[4] Donaldson, H. H., "The Rat" (Philadelphia) 1924.

3826

Nature of Pigment in Intestine of the Marine Worm Chaetopterus.

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From the Mayo Foundation, Rochester, Minn.

While at Woods Hole one of us (Alvarez) became interested in the green color of the intestine of the otherwise white *Chaetopterus*. On turning to the article by Enders[1] it was found that doubt still exists as to the nature of the pigment: some have thought it to be biliverdin, others have assumed it to be chlorophyll, and others have been so sure that it is not chlorophyll that they have dignified it with a special name, chaetopterin. It seemed worth while to settle the question because if the pigment should be biliverdin, the animal might be valuable for studies on bile formation.

The digestive tracts from several worms were accordingly extracted with alcohol and determination made by one of us (Sheard) of the percentage transmission of light of various wave lengths (from 700 mμ to 430 mμ) with a Keuffel and Esser color analyzer (spectrophotometer). The transmission curves (Fig. 1) obtained from the unknown pigment and from solutions of chlorophyll from the fresh stems of buckwheat, leaves of bean plants and grass were so similar as to warrant the statement that the absorption bands (zones) were produced in all cases by the same pigment or pigments, that is, by chlorophyll *a* or chlorophyll *b* or both. The most pronounced absorption zone in an alcoholic solution of chlorophyll has its maximal point at about 660 mμ. This band is common to all the curves of Fig. 1 irrespective of the source of the coloring material or degree of dilution of the solution. Curve 2 was obtained from an alcoholic extract of the stems of buckwheat, the solution being of such a concentration as to reproduce as nearly as possible Curve 1 (alcoholic extract of chaetopterus), both in respect to the number of absorption zones and their degree of absorption. Curve 3, obtained with a highly dilute alcoholic extract of fresh leaves from a bean plant, is included to show the marked effects of dilution of the solutions on the spectrophotometric curves. Extracts of chaetopterus and of buckwheat, when sufficiently diluted, gave spectrophotometric curves similar to Curve 3, that is, with pronounced absorption zones in the orange-red and blue-violet, but none in between.

On making a histologic study of the material it was found by one of us (Higgins) that the green matter had permeated the tissues to such an extent that it was hard to be sure of its original site. How-

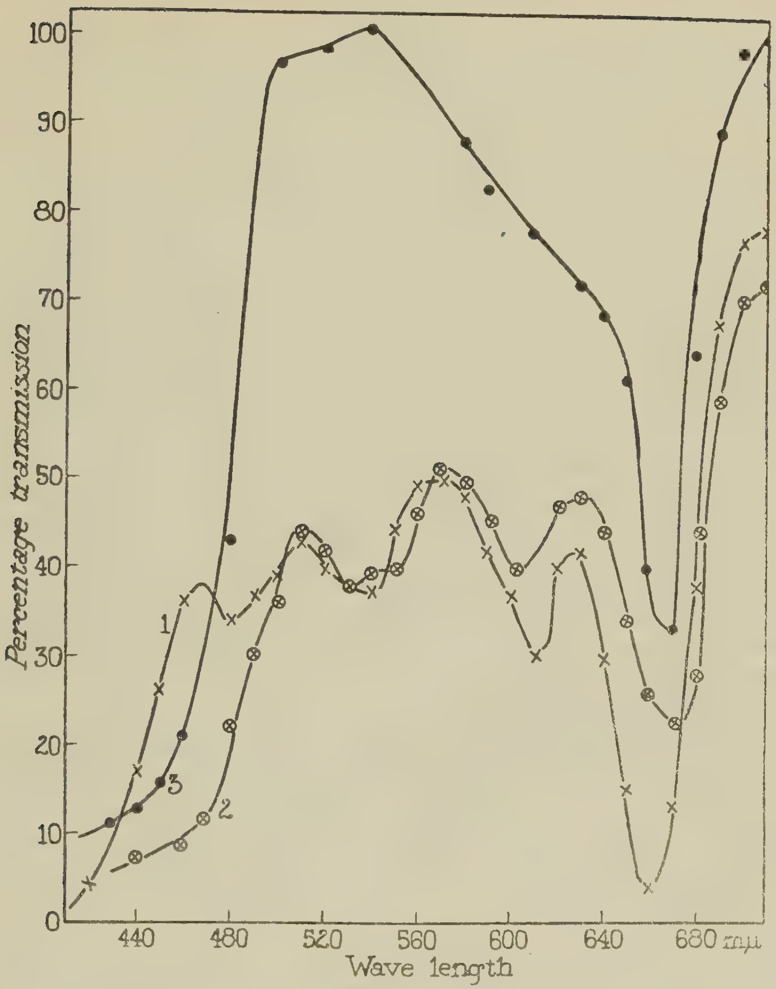

FIG. 1.

Spectrophotometric determinations of alcoholic extracts of (1) chaetopterus, (2) buckwheat, (3) bean.

ever, scattered through several fields there were single cells resembling protophytes, and in many places, there were what appeared to be large colonies of the organism. These invaders were not always within the tissues but sometimes were in the lumen of the bowel where they may have been thrown by the disintegration of some of the cells. The impression left was that the green structures are primitive plants living in symbiotic relationship with the worm, and this agrees with the conclusion of others who have studied the problem.[2]

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[1] Enders, H. E., *J. Morphol.*, 1909, xx, 479.

[2] Geddes, Patrick, *Quart. J. Microsc. Sc.*, 1879, xix, 434.

3827

Relation Between Cardiac Size and Cardiac Output Per Minute Following Administration of Digitalis.

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Recently Harrison and Leonard[1] have studied the effect of injecting digitalis upon the cardiac output in normal dogs, and found that it diminished. From these experiments they drew the inference that digitalis decreased cardiac output also in patients suffering from heart failure and that its beneficial effect in them was due to this action. It seemed to us that this inference was not justified unless the effect of digitalis upon the size of the heart was also studied.

In making our experiments in dogs the cardiac output was estimated according to the Fick[2] principle. Samples of arterial blood were taken from a femoral artery and those of mixed venous blood from the right ventricle by means of a special cannula inserted into that chamber through the right external jugular vein.[3] The oxygen consumption was measured with a Benedict spirometer equipped with a graphic recording drum. All the dogs were trained in order to accustom them to these procedures.

Tincture of digitalis (Upsher Smith) was injected intravenously. We injected 25 to 30% of the calculated lethal dose for cats which has been regarded as equivalent to the therapeutic dose in man.[4] This dose is multiplied by 1.16 to arrive at a comparable quantity for dogs.[5] Roentgenograms were taken whenever the cardiac output was measured.

TABLE I.

Effect of injection of digitalis in dogs in respect to cardiac output and cardiac size.

Dog Wgt.	Time with reference to digitalis injection	Cardiac output	Cardiac output, % of initial	Heart area	Heart area % of initial	Digitalis
Kg.		cc. per minute	%	sq. cm.	%	cc.
12.5	Before	4285	100	43.6	100	3.0
	2 hrs. after	2344	55	35.7	82	
	1 day after	2627	61	38.6	89	
	5 days after	4823	112	42.3	99	
14.8	Before	4066	100	44.7	100	3.3
	2 hrs. after	2193	54	35.7	80	
	1 day after	2592	64	37.2	83	
	5 days after	3267	80	41.8	94	

In Table I are shown data from experiments in 2 dogs. These are typical of all our results. There was a decrease in cardiac output after the administration of digitalis with a return toward normal in 5 days. There were also decreases in the size of the heart. These changes in the size of the heart paralleled the changes in cardiac output, that is to say, with decrease in output there was decrease in size. This result is the same as that found by Harrison and Leonard so far as cardiac output is concerned. We injected digifolin in one of our experiments in order to make our results comparable with theirs. The decrease in cardiac output and size of heart was always present 2 hours after the administration of digitalis and occurred irrespective of the heart rate which was sometimes increased and sometimes decreased.

The per cent decreases in cardiac output are greater than the per cent decreases in heart area. This is, of course, expected for the output is a cubic while the area is a square measurement.

We conclude that there is close correlation between volume output and cardiac size in the same dog. When the output decreases this results from the effect of digitalis on the dimensions of the heart, in this case the dimension in the frontal plane. In patients with heart failure a similar comparison between size and output should be instituted; but in this case the relation of length of cardiac muscle fiber to optimum length must no doubt be taken into consideration.

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[1] Harrison, T. R., and Leonard, B. W., *J. Clin. Invest.*, 1926, iii, 1.

[2] Fick, A., *Verhandl. physik.-med. Ges. Würzburg.* 1870.

[3] Stewart, H. J., *J. Biol. Chem.*, 1925, lxii, 641.

[4] Robinson, G. C., and Wilson, F. N., *J. Pharmacol. and Exp. Therap.*, 1918, x, 491.

[5] Cohn, A. E., unpublished experiments.

3828

Physiological Action of Cyanide on Protoplasm.

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From the Zoological Laboratory, University of Pennsylvania.

Since it is known that hydrogen cyanide passes through living membranes very rapidly,[1] it was thought desirable to ascertain whether the toxicity of cyanide is due to its action on the internal protoplasm or to its effect on the cell membrane. In this study a small species of amoeba of the proteus group was used. Some individuals were immersed in aqueous solutions of hydrogen cyanide or potassium cyanide and others were injected with the same solutions by means of the Chamber's micro-manipulator. The concentrations of cyanide ranged from N/100 to N/3000 and the pH values used were 6.8 and 7.4. The animals were studied under direct-illuminated and dark-field microscopes and the rate of Brownian movement was used as an index of viscosity of the protoplasm.

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* National Research Fellow in Biological Sciences.

By means of a micro-pipette, aqueous solutions of HCN and KCN were injected into the amoeba in amounts equal to nearly one half the volume of the animal; the cyanide quickly diffused throughout the protoplasm, producing a decrease in viscosity, as was evident by an increase in the rate of Brownian movement. The organisms recovered and no toxic effect was noticeable. The action of injected cyanide on the protoplasm is not greatly different from that of injected distilled water.

When amoeba are immersed in aqueous solutions of HCN or KCN, the viscosity of the protoplasm is decreased and the animal gradually swells, assuming a spherical form, and Brownian movement within the internal protoplasm is greatly accelerated, indicating a liquefaction. Finally, the cell membrane ruptures and disintegration of the cell occurs. HCN is much more powerful in its action than KCN, even when the solutions are of the same pH value. It is suggested that the swelling of the organism in cyanide is due to an increase in permeability of the cell membrane to water and a simultaneous decrease in viscosity of the protoplasm.

If amoeba are immersed in aqueous solutions of HCN or KCN and the cell membrane torn with micro-dissection needles, the protoplasm flows from the torn surface and a new membrane is formed around the extruded globule of protoplasm, indicating that the internal protoplasm is not injured. The reaction of these individuals is similar to the reaction of those placed or immersed in HCN or KCN. The membrane seems to be stronger and firmer after the animals are immersed in KCN than when they are placed in HCN.

These experiments seem to indicate that cyanides have little, if any, toxic effect on the interior protoplasm of amoeba but are decidedly toxic to the membrane when applied to the surface of the cell.

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[1] **Brinley, F. J., *Biol. Bull.*, 1927, liii, 5.**

3829

A Relationship Between Phosphorus, Creatinine and Acidity in Urinary Excretion.*

GILBERT J. RICH. (Introduced by Herman M. Adler.)

From the Illinois Institute for Juvenile Research.

The data in this study were obtained from 171 24-hour specimens of urine furnished by 57 university students, each of whom gave three such samples at intervals of about one week, and who did not control either their diet or their muscular activity. Every specimen of urine was analyzed for its content of phosphorus (inorganic and easily hydrolysable) and creatinine and its total titratable acidity (including the formol titration).

When any pair of the above determinations is plotted graphically, there is a marked tendency for the 2 to vary concomitantly from specimen to specimen, so that the values cluster about a straight line save for a few divergences. This tendency is best expressed in the statistical coefficient of correlation used in biometric and psychometric work. The coefficients of correlation between each of the three pairs of chemical determinations are given in the accompanying table under the head of "Total Correlation." The size of the coefficients, together with the fact they are obtained upon as many as 171 samples, is such as to make them statistically significant and beyond any reasonable probability that they occurred by chance.

Intercorrelations—171 24-hour Specimens.

	Total correlation	Correlation with remaining factor "partialed" out
Total Acid—Creatinine	+.70	+.50
Creatinine—Phosphorus	+.64	+.37
Total Acid—Phosphorus	+.62	+.32

With interrelationships of this type, the correlation between any two measures may depend upon their common relation to a third measure. This dependence may be eliminated in any given case by obtaining the partial correlation, that is, the correlation between the 2 measures with the third one held contant or "partialed" out. These values are shown in the last column of the table, where, for example, the correlation between total acidity and creatinine is computed with phosphorus held constant.

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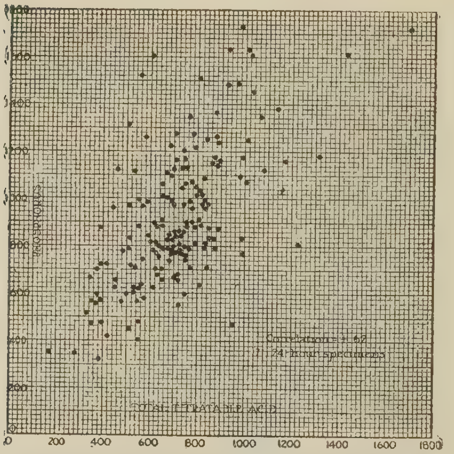
* Laboratory facilities and advice furnished by Prof. F. C. Koch of the University of Chicago are gratefully acknowledged.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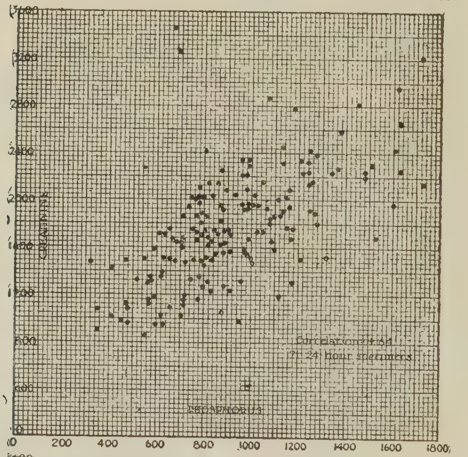
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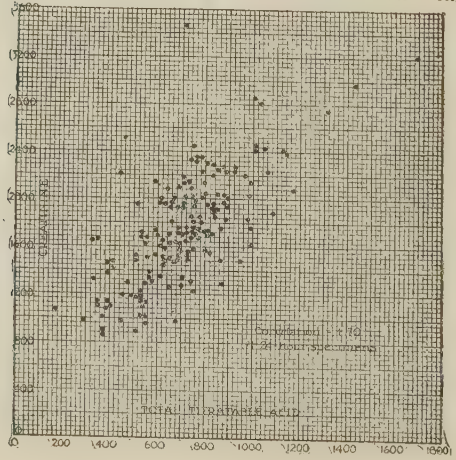
CREATININE
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1800

It will be noted that the correlation between the total titratable acid and the creatinine content of the urine is the highest, and is relatively independent of concomitant variation with the phosphorus content. This means that, under diverse conditions, there is a marked tendency for these two elements in the urine to be either high or low at the same time. No explanation of this relationship is apparent at the present time. While, on the other hand, it is generally accepted that the acidity of urine depends largely upon the phosphoric acids that it contains, the correlation between acidity and phosphorus content is the lowest of the three and is in large measure dependent upon a concomitant variation in the creatinine of the urine. Moreover, there is a significant relationship between amounts of phosphorus and of creatinine in the urine. This relationship must be considered in the light of the evidence adduced by Fiske and Subbarow[1] that the creatine in voluntary muscle exists in the form of phospho-creatine, the presence of whose metabolic products in urine might in part explain our findings.

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[1] Fiske, C. H., and Subbarow, Y., *Science*, 1927, lxv, 401.

3830

Effect of Diets High in Protein but Inadequate in Calories on Weights of Obese Patients.

ROBERT W. KEETON AND HELEN MAC KENZIE.

From the Department of Medicine, University of Illinois, College of Medicine.

A patient (L. W.), female, weight 277 lbs., height 63.2 in., surface area 2.25 sq. m., age 21, with basal requirement of 2,000 calories was hospitalized and placed on a diet containing 90 gm. of protein and an inadequate number of calories varying between 802 and 1027. Over a period of 7 months, from Nov. 1, 1925, to June 1, 1926, she lost 65 pounds. All of this time the patient was allowed opportunity for daily walks and during a portion of the period she was given routine light work about the hospital. Then a second period followed in which she lived at home, weighed her food daily, but was actively working. At the opening of this period, June 1, 1926, she weighed 212 pounds and at the close, Feb. 10, 1907, 205 pounds. Thus her weight over this period of 8 months was stationary. Her basal metabolic rates were taken at regular intervals during this reducing period and found to be normal. Her nitrogen excretion showed a slight positive balance, indicating at least a nitrogen equilibrium. This patient was always found to be trustworthy. She was so anxious to lose weight that at times difficulty was experienced in making her eat all the food on her trays. In the hospital the intake did not exceed that shown by the figures. During the eight months when she was not in the hospital she reported at regular intervals and stated that she was following closely the diet. I feel that while she is to be trusted there was more opportunity for error during this period.

A second patient (L. S.), female, weight 292.5 lbs., height 67.5 in., surface area 2.4 sq. m., age 26, basal requirements 2,140 calories, was placed on diets deficient in calories, basal 2,140; basal —30%, 1,500; basal —46%, 1,160; which however contained 120 gm. of protein. She lost weight very slowly from 292.5 lbs. to 260 lbs. over a period of 172 days. At one time her weight remained practically stationary for 50 days on a basal —30% diet (1500 calories).

Her basal metabolic rates also remained normal in the face of this severe undernutrition. She too remained in nitrogen equilibrium and at times appeared to be actively storing nitrogen. This second patient is a well educated young woman, of German extraction, and a teacher in the City Schools of Chicago. She was fully acquainted with the significance of the experiment and the necessity for accuracy. She showed on many occasions her willingness to cooperate when this cooperation involved a distinct sacrifice. I feel that her statements are to be trusted absolutely. This maintenance of nitrogen equilibrium and tendency to store nitrogen has been found present in 3 other female obese patients who were on diets containing 90 gm. of protein and approximately 1000 calories.

3831

Blood Lactic Acid in Relation to Metabolic Rate.

L. E. ADAMS AND G. L. FOSTER.

From the Division of Biochemistry and Pharmacology, University of California Medical School, Berkeley, and the Department of Chemistry, Northwestern University Medical School, Chicago.

The newer physiology of muscle contraction leads one to the hypothesis that, at least in muscle, it is the rate of glycolysis (or anaerobic cleavage of sugar with production of lactic acid) which determines the rate of respiration (oxygen absorption) of the tissue.[1, 2, 3] It would be interesting to know how far one could account for conditions of increased metabolism of the whole organism, other than in muscular exercise, on the basis of increased glycolysis by the tissues and hence, increased oxidation to keep pace therewith. For later researches by Meyerhof[4] and Warburg[5] and their collaborators show that the glycogen, lactic acid, oxygen relationship is not peculiar to muscle tissue but should be regarded rather as an attribute of all living cells. At least, the quantitative relation between oxygen absorption and lactic acid removal is the same for all cells and tissues hitherto examined, *i. e.,* Lactic acid removed/Lactic acid oxidized = 4/1 to 5/1, which is at least a broad hint of the fundamental identity of the energy-yielding reactions in all forms.

We have examined the concentration of lactic acid in the blood of man and animals under several conditions of increased or decreased metabolism. It is conceivable that although respiration may keep pace with glycolysis and prevent accumulation of lactic acid, nevertheless, in a case of increased rate of glycolysis, equilibrium might be reached at a higher concentration of lactic acid, which might be demonstrable by blood analysis. In man, we have increased the metabolism by large feeding of meat; while with rabbits we have used thyroid feeding to increase the metabolic rate and thyroidectomy to reduce it.

The lactic acid determinations were made by the Clausen[6] method, with substantially the same modifications as those suggested by Brehme and Brahdy.[7] Oxygen absorption was measured by the McKesson modification of Krogh's apparatus in the experiments on man, while the apparatus of Foster and Sundstroem[8] was used for the rabbits.

Meat Ingestion. The experimental subjects (healthy young men in the postabsorptive state) lay at complete muscular rest for 40 to 50 minutes before the basal oxygen measurement and blood sample. Blood was taken from an arm vein with little or no stasis. Five hundred gm. of cooked lean beef were fed and observations made at intervals thereafter, the subject lying quietly throughout, and especially quiet for 30 minutes preceding each sample or measurement.

Ingestion of the meat caused in all cases a marked increase in metabolism. In no case did the blood lactic acid increase. These experiments show that increases in metabolism of 25 to 30% above the basal level caused by meat ingestion are not attended with any demonstrable rise in lactic acid.

Thyroidectomy and Thyroid Feeding. Young rabbits weighing about 1.5 kg. were completely thyroidectomized. Three or 4 weeks after the operation observations of blood lactic acid and basal metabolism were made. Great care was taken to exclude muscular activity before taking the blood samples or while measuring oxygen absorption.

In no case was there any significant change in lactic acid although thyroidectomy caused a 25% decrease in the basal metabolism, and thyroid feeding of the operated animals caused approximately 100% increase above the cretinoid level which existed before thyroid administration was begun.

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[1] Meyerhof, O., *Pflug. Arch.*, 1921, clxxxviii, 112.

[2] Meyerhof, O., Lohmann, K., and Meier, R., *Biochem. Zeitschr.*, 1925, clvii, 473.

[3] Hill, A. V., Long, C. N. H., and Lupton, H., *Proc. Roy. Soc.*, 1924, 97B, 84 and 155.

[4] Meyerhof, O., *Biochem. Zeitschr.*, 1925, clxii, 43.

[5] Warburg, O., Posener, K., and Negelein, E., *Biochem. Zeitschr.*, 1924, clxx, 309.

[6] Clausen, S. W., *J. Biol. Chem.*, 1922, lii, 263.

[7] Brehme, Th., and Brahdy, B., *Biochem. Zeitschr.*, 1926, clxxv, 348.

[8] Foster, G. L., and Sundstroem, E. S., *J. Biol. Chem.*, 1926, lxix, 565.

3832

General Tuberculin-like Reactions in Rheumatic Fever Patients Following Intravenous Injection of Streptococcus Vaccines or Nucleoproteins.

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From the Hospital of the Rockefeller Institute for Medical Research.

Several years ago one of us[1] suggested that rheumatic fever had many points of similarity with tuberculosis. Since then we[2, 3] have shown that rabbits may be rendered hypersensitive to streptococci by the production of focal lesions with various types of non-hemolytic streptococci which have been isolated from patients with rheumatic fever and other diseases. As these streptococci have been shown[4] to belong to various immunological groups, we formulated the hypothesis that if they had any etiologic relationship with the disease it was because of a peculiar type of tissue reactivity acquired by the rheumatic patient. We have, therefore, tested the reactivity of a number of patients in the late stages of rheumatic fever to intravenous injection of streptococci or products derived from them. These test materials were heat killed suspensions, vaccines, prepared from both green and hemolytic streptococci and nucleoproteins made from hemolytic streptococci. In all instances the initial dosage was very small and subsequently increased by slow degrees in order to avoid too severe reactions.

The type of febrile reaction has been uniform, and consisted, as a rule, of slowly rising temperature which usually did not begin until 6 to 8 hours after the injection, and often did not reach its maximum until from 16 to 30 hours. In a few instances the fever has persisted for 2 to 4 days, but as a rule it has disappeared by the end of 36 hours. There was an accompanying increase in pulse rate, often headache, dizziness and general malaise. Chills or chilly sensations have been rare. In some cases there has been moderate reactivation of previously quiescent arthritis, and symptoms pointing to activation of cardiac foci.

These reactions are strongly suggestive of the late reactions observed in tuberculous individuals after injections of tuberculin. They are distinctly different from the non-specific shock reactions we have observed in control cases and that others have reported in rheumatic patients following intravenous injection of typhoid vaccine. In the latter instance the reaction is usually ushered in by a chill within 1 to 4 hours after the injection; this is followed within 1 to 3 hours by the peak of the fever, which soon subsides. In our cases the reaction following typhoid vaccine was usually completed in 10 hours or less.

Small[5] has observed similar late general reactions 24 to 48 hours after subcutaneous injection of rheumatic fever patients with vaccines of *Streptococcus cardioarthritidis* described by him. At times these reactions were very severe. In their focal features they were strongly suggestive of tuberculin-like reactions.

In view of the rather widespread variety of streptococci with which it has been possible to induce this reaction in rheumatic fever patients, and also because of the peculiar character of the general and focal reaction which follows injection of these streptococci or their products—a reaction similar to that induced by tuberculin in tuberculous subjects—we feel justified in assuming that these patients have a tuberculin-like allergy (hyperergy) towards streptococci, and that probably this altered tissue reaction accounts for many of the peculiar characters of this disease.

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[1] Swift, Homer F., *Am. J. Med. Sc.*, 1925, clxx, 631.

[2] Andrewes, C. H., Derick, C. L., and Swift, Homer F., *J. Exp. Med.*, 1926, xliv, 35.

[3] Derick, C. L., and Swift, Homer F., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 222.

[4] Swift, Homer F., and Kinsella, R. A., *Arch. Int. Med.*, 1917, xix, 381.

[5] Small, J. C., personal communication, and paper read before Phil. Path. Soc., Dec. 8, 1927.

Southern Branch.

Tulane University, December 13, 1927.

3833

Experimental Chronic Pericarditis. Further Contribution to the Study of Cardiac Hypertrophy.

GEORGE R. HERRMANN AND JOHN H. MUSSER.

From the Department of Medicine, School of Medicine, Tulane University.

Within the past year and a half we have performed periocardotomies on 60 dogs through the more or less avascular midsternal route and have introduced into the pericardium various substances, as bacterial antigen, organic and inorganic compounds. Morphine and ether anesthesia by the intratracheal method with artificial respiration were employed. Electrocardiograms and roentgenograms were made before and at intervals after operation and just before death when it was possible to do so. At autopsy the sternum, with the anterior mediastinum, the pericardium, heart and lungs intact, was carefully removed together and subsequently dissected and weighed.

Six dogs died at the end of the first week with serous, fibrinous, and purulent pericardial effusion and with conspicuous anterior mediastinitis from the sternal wound to the heart. The heart weight-body weight ratios of these dogs ranged from 0.00988 to 0.00812, and were, thus, below the maximum normal of 0.01000, but above the average of 0.00798 established in a series of 200 normal dogs of all mongrel breeds, of various ages, sexes, and sizes.[1]

One dog survived for 200 days with thick, anterior mediastinal bands of adhesions, but without any visceral adhesions. In this instance the heart weight-body weight ratio was found to be 0.00916. Another dog with a pure anterior mediastinitis for 10 days had a H. W./B. W. ratio of 0.00914. Five dogs had partial visceral synechia as well as heavy anterior mediastinal bands of adhesions for 212, 108, 83, 81 and 43 days, and these presented, respectively, H. W./B. W. ratios of 0.0133, 0.0100, 0.0131, 0.0079, 0.0082; thus

TABLES.

Anterior Mediastinitis with Purulent Pericarditis.

No.	Days	Ratio
106	8	0.00988
119	8	0.00812
123	6	0.00880
137	6	0.00854
138	6	0.00860
140	4	0.00886

Partial Synechia with Chronic Adhesive Mediastinitis.

No.	Days	Ratio
130	212	0.01330
147	108	0.01000
149	83	0.01310
132	81	0.00790
125	43	0.00956

Anterior Mediastinitis Chronic Without Visceral Adhesions.

No.	Days	Ratio
128	200(?)	0.00916
122	11	0.00914

Complete Synechia with Chronic Anterior Mediastinitis.

No.	Days	Ratio
150	252	0.01420
156	221	0.0133
148	116	0.0117
146	111	0.0114
145	97	0.0091
131	62	0.01030
140	45	0.01000
115	42	0.01180
135	33	0.0090
133	14	0.01000

presenting evidences of some cardiac hypertrophy, especially in dogs with the more extensive lesions for longer periods of time.

The dogs had complete synechia of the pericardium as well as anterior mediastinal anchorage for periods of 252, 220, 116, 111, 97, 62, 45, 42, 33, and 14 days, and these presented H. W./B. W. ratios of 0.0142, 0.0133, 0.0117, 0.0114, 0.0091, 0.0103, 0.0100, 0.018, 0.0090, and 0.01000, respectively. Thus, with two exceptions, there is evidence of considerable hypertrophy of the heart as a result of the experimentally produced chronic adhesive mediastinopericarditis. Pathologically increased work, of course, suggests itself as the etiological factor of the hypertrophy.

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[1] Herrmann, G. R., *Am. Heart J.*, 1925, i, 2.

3834

Blood Volume Changes in Carbon Arc Irradiation.*

H. S. MAYERSON. (Introduced by Henry Laurens.)

From the Laboratory of Physiology, Tulane University, School of Medicine.

In previous communications[1, 2, 3] evidence was reported suggesting an increase in blood volume following carbon arc irradiation, which dilution was believed to mask actual increases in the physical and chemical blood constituents. In the present study we sought to

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* Aided by a grant from the David Trautman Schwartz Research Fund.

determine the nature and extent of these changes in plasma and red cell volume.

Five short-haired, white dogs were irradiated abdominally with the "Pan Ray" carbon arc with dosages of 70 to 83 g. cal. per sq. cm. at intervals varying from 3 hours to 6 days for a total of 15 separate exposures. Total plasma volume was obtained by the dye method, using brilliant vital red; specific gravity by the falling drop method of Barbour and Hamilton; Hb by the Newcomer method and hematocrit according to Van Allen. Total cell volume was calculated indirectly from the hematocrit reading. The results, with minor exceptions, are uniform and confirm the previous evidence that the primary result of irradiation is an increase in plasma volume of 6 to 37%, depending on the dosage and the interval between successive exposures. The dilution usually reaches a maximum in each animal which is maintained but not exceeded by subsequent irradiation, except when massive exposures are given. In 2 such experiments where the animals were irradiated twice for 53 minutes at 80 cm. (each dose equivalent to 83 g. cal. per sq. cm.) with an interval of 3 hours between exposures, there was evidence of slight concentration at the end of the second irradiation following the usual previous dilution. Red cell destruction is frequently observed in preparations made immediately following massive irradiation. The increase in red cell number, volume, and Hb previously reported is independent of the blood volume changes and indicates a real stimulus to the hematopoetic organs as a result of the irradiation.

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[1] Miles, A. L., and Laurens, H., *Am. J. Physiol.*, 1926, lxxv, 462.

[2] Mayerson, H. S., *Am. J. Physiol.*, 1927, lxxxi, 686.

[3] Mayerson, H. S., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 882.

3835

Ratio of Urea Nitrogen to Total Non-Protein Nitrogen in the Blood in Pregnancy.

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From the Department of Bio-Chemistry and of Obstetrics of the School of Medicine, Tulane University.

In normal whole blood the urea nitrogen usually accounts for about 50% of the total non-protein nitrogen, although the variations found in this ratio (35-55)[1] scarcely justify the practice occasionally seen in hospital laboratories by which the concentration of urea nitrogen is calculated from the non-protein nitrogen or vice versa.

The fact seems well established that in the blood of patients with nitrogen retention of considerable magnitude the relative concentration of urea nitrogen may be increased as high as 75% of the non-protein nitrogen.[2] On the other hand, the finding of a lowered ratio to non-protein nitrogen which has been noted by certain investigators to occur in the blood of pregnant women has not received universal confirmation, for while certain observations would indicate that this lowered ratio is found in the blood in normal pregnancy,[3] others have found this relative decrease in the urea fraction only in abnormal cases.[4] The records of still other observations would indicate that the ratio of urea to non-protein nitrogen is the same in the blood of non-pregnant and of pregnant subjects.

We have carried out a series of observations concerning the ratio urea nitrogen: total non-protein nitrogen and for this purpose have analyzed 316 samples of blood taken from 162 normal pregnant women, from the first to the ninth month of gestation, 55 samples of blood taken from 55 women from 11 hours to 5 months after delivery, and (as a check on our analytical technique) 14 samples of blood from 14 non-pregnant individuals, 4 women *(nullipara)* and 10 men. The determination of urea and of non-protein nitrogen were made by the methods of Folin and Wu[6] on oxalated blood. The results may be summarized as follows:

Ratio of urea-nitrogen to non-protein nitrogen in the blood—

	Average	Max.	Min.
In 162 normal pregnant women	33.1	52.0	22.2
In 55 women 11 hrs. to 5 months after normal delivery	42.6	51.1	27.8
In 4 normal non-pregnant women	45.6	53.1	42.5
In 10 normal men	48.5	56.1	44.5

The above results would appear to indicate that the per cent of non-protein nitrogen present as urea nitrogen is lower in the blood of normal pregnant women than in that of non-pregnant individuals. In a few cases we have been able to obtain monthly samples of blood during all or a considerable portion of the period of gestation and have been able to observe the gradual decrease in the ratio during the later months of pregnancy.

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[1] Folin, O., *Physiol. Rev.*, 1922, ii, 469.

[2] Myers, Victor C., "Practical Chemical Analysis of Blood," 1924.

[3] Folin, O., Mellon Lecture, University of Pittsburgh, 1917; Stander, H. J., *Bull. Johns Hopkins Hosp.*, 1924, xxxv, 133.

[4] Killian, J. A., and Sherwin, C. P., *Am. J. Obst. and Gynecol.*, 1921, ii; Caldwell, W. E., and Lyle, W. G., *Am. J. Obst. and Gynecol.*, 1921, ii; Harding, V. J., and Drew, K., *J. Obst. and Gynecol. of the Brit. Emp.*, 1923, xxx, 4; Harding, V. J., Allen, K. D., and Van Wyck, H. B., *J. Obst. and Gynecol. of the Brit. Emp.*, 1924, xxxi, 4.

[5] Plass, E. D., *J. Biol. Chem.*, 1923, lvi, 17; *Bull. Johns Hopkins Hosp.*, 1924, xxxv, 345.

[6] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii, 81.

3836

A Differential Plating Medium for Lipase-Producing Bacteria.*

R. H. TURNER. (Introduced by J. H. Musser.)

From the Department of Medicine, Tulane University School of Medicine.

A need for a differential plating medium for lipase-producing bacteria was felt when a study was begun a year and a half ago for the purpose of classifying the bacterial flora of the gastro-intestinal canal in certain diseases possibly due to bacteria present there; *i. e.*, pellagra, sprue and pernicious anemia. Since diet has such an important bearing upon these diseases, it was decided to try as a basis for classification, in part at least, the action of the various bacteria present on certain fundamental elements of food, *i. e.*, fats, starch, protein. This seems practicable only by means of differential plating media.

The medium under discussion has been in use for more than a year and only a partial study of its possibilities has been made. However, since it might be useful to others, it was thought well to publish a description at this time. The composition of the media is as follows: Sugar free meat digest fluid, 1,000 cc.; Di-basic sodium phosphate, 5 gm.; Agar, 30 gm. Melt, clear, adjust for final pH of 7.6. Autoclave. Add 0.125 gm. nile blue sulphate in 100 cc. 25% ethyl alcohol. Tube in 6 to 7 cc. amounts in tubes already sterile. Arnold for 15 minutes. Store. To use, the agar is melted and cooled to 45°-50° C. A sterile emulsion of fat is added to about 10% volume; 0.7 cc. for 7 cc. tube. We use either cream or one of cotton seed oil made according to directions in U. S. Pharmacopeia for codliver oil except 25% more water is used than prescribed. The

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* Aided by grants from the Schwartz Research Fund and the Committee on Scientific Research of the American Medical Association.

inoculum and emulsion are thoroughly mixed with agar, poured into a petri dish, and allowed to harden. Incubation has been in the anaerobic jar, the air of which has been exhausted by burning phosphorus. Twenty-four hours is usually sufficient to bring out the typical colony. Surface streaked plates are not so satisfactory as plates that are inoculated and then poured, since the surface colony does not always give the characteristic picture.

The lipase-producing colony is identified by the dark blue zone surrounding it and is in striking contrast to the dull bluish gray color of the remainder of the plate. The colony itself is usually colorless. The zones are usually of greater diameter than the colonies except in those colonies with marked tendency to spread. When examined with the low power microscope, the zone is seen to be made up of tiny blue globules or shapeless masses of the same color. None of the colorless globules of oil seen elsewhere through the plate is observed in the zone. If the colony under study is well separated from others, a faded circular zone 2-3 mm. wide will be observed surrounding the blue zone. (This is probably due to diffusion of dye toward the colony to take the place of that taken up by the fatty acids and soaps.)

In studies on gastro-intestinal contents of some 15 patients, 10 strains of bacteria producing the deep blue zone have been isolated in pure culture. When these were grown in sugar-free meat infusion broth plus cotton seed oil emulsion, evidences of digestion of fat were noted. One strain, which has since been lost, would, in 2 or 3 days, change a layer of emulsion 3 mm. thick into a fluffy layer 8 mm. thick. After this change the material would stain deep blue with nile blue sulphate, which it would not do before. As to the other strains, the results are not so striking, but gross evidences of fat splitting are obvious. From cultures showing either marked or slight gross change in the fat layer, fatty acids or soap may be obtained by extracting with ether from an alkaline solution, then precipitation by hydrochloric acid.

No organism giving the deep blue zone described has been found without marked lipolytic action. However, 3 organisms have been isolated which gave a transient purple zone and which in pure culture in liquid media produced no lipase. On removal of the plates from the anaerobic jar, these would show only a faded area about the colony. After standing an hour, a pale purple haze develops, the colony being unstained. This color is localized to the tiny globules. It lasts only 2 to 3 hours.

As is well known, certain bacteria, especially anaerobes, possess the power of permanently decolorizing practically any of the analine dyes. If colonies of this type are closely placed on the plate, the lipase producing colony will not be detected. However, if the colonies are spaced as much as 3 or 4 mm. apart, it is not interfered with. The plate may be crowded with thousands of colonies of weak or no reducing power, without interfering with the production of the typical blue zone.

A possible source of error in considering a colony as a lipase producer, which it is not, lies in the fact that certain bacterial colonies themselves will take the stain. It is then necessary to distinguish between a blue colony and a blue zone, which is usually not difficult.

ERRATUM—Page 21—*B. enteritides* should read *B. enteritidis*.

NOTICE TO AUTHORS.

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